Genetic Influences on Memory Performance, Memory-Related Brain Activity, and Neuroanatomy Investigated with Functional and Structural Magnetic Resonance Imaging

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Dedicated to Jeannine
and to my Family

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Abbreviations

5HT	5-hydroxytryptamine (Serotonin)
AD	Alzheimer's Disease
apoE	Apolipoprotein E
APOE	Apolipoprotein E gene
BDNF	Brain derived neurotrophic factor
BOLD	Blood oxygen level dependent
cAMP	Cyclic adenosine monophosphate
CERAD	Consortium to Establish a Registry for Alzheimer's Disease
CNS	Central nervous system
CREB	cAMP-response element binding protein
FAD	Familial Alzheimer's Disease
FDG	18F-2-deoxy-2-fluoro-D-glucose
fMRI	functional magnetic resonance imaging
His	Histine
LTP	Long term potentiation
MCI	Mild cognitive impairment
Met	Methionine
MTL	Medial temporal lobe
PET	Positron emission tomography
PRNP	Prion protein gene
PS1	Presenilin 1 protein
PSEN1	Presenilin 1 gene
SAD	Sporadic Alzheimer's Disease
SNP	Single nucleotide polymorphism
SPECT	Single photon emission computed tomography
Tyr	Tyrosine
Val	Valine

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Zusammenfassung

Gegenstand aktueller Debatten ist häufig die Frage, zu welchem Ausmass verschiedene Domänen der menschlichen Kognition, wie zum Beispiel das Gedächtnis, von unseren Genen und/oder von unserer Umwelt beeinflusst werden. In Zwillingsstudien konnte eine ungefähre Heritabilität von 50% festgelegt werden. Somit wurde aufgezeigt, dass natürlich auftretende genetische Variationen einen wichtigen Einfluss auf die interindividuelle Variabilität in den Gedächtnisleistungen ausüben. In dieser Doktorarbeit wurden genetische Analysen, die funktionelle und strukturelle Bildgebung des Gehirns und quantitative neuropsychologische Methoden kombiniert, um den Einfluss der beiden Gene Apolipoprotein E (APOE) und Presenilin 1 (PSEN1) auf die normalen Gedächtnisleistungen, auf gedächtnisbezogene Hirnaktivierungen und auf die Neuroanatomie zu untersuchen.

In der ersten Studie versuchten wir herauszufinden, ob der APOE Genotyp eine Rolle in der normalen Gedächtnisfunktion spielt. Das APOE Gen hat drei Allelvarianten: ε2, ε3 und ε4. Das ε4 Allel erhöht das Risiko an der sporadischen Form der Alzheimer Demenz (AD) mit spätem Beginn zu erkranken. In Personen mittleren Alters und in älteren Personen wurde gezeigt, dass das ε4 Allel schädliche Auswirkungen auf kognitive Leistungen, auf die gedächtnisbezogene Hirnaktivität und auf den Glukose Metabolismus des Gehirns ausüben kann. Bisher wurde jedoch in Unabhängigkeit der Alzheimer-bezogenen Pathologie kein Einfluss des ε4 Allels auf das normale Gedächtnis berichtet.
In einer Stichprobe von 340 jungen und gesunden Personen fanden wir einen besseren episodischen Gedächtnisabruf in ε4 Allel Trägern, wenn diese mit ε2 oder ε3 Trägern verglichen wurden. Weiter untersuchten wir die Hirnaktivität und Hirnanatomie von 34 Versuchspersonen mittels funktioneller und struktureller Magnet Resonanz Tomographie. Dabei konnte ein genetischer Effekt auf die gedächtnisbezogene Hirnaktivität gezeigt werden. In den ε4 Trägern nahm die Hirnaktivität in einem gedächtnisrelevanten Netzwerk während des wiederholten Lernens visueller Reize ab. Ausserdem wiesen die ε4 Träger eine reduzierte Hirnaktivität beim Erinnern der zuvor gelernten Reize auf. Die ε2 und ε3 Träger hingegen zeigten eine Aktivitätszunahme während des Lernens und eine erweiterte Aktivierung während des Erinnerns. Diese genotypinduzierten Hirnaktivierungsunterschiede traten auf, obwohl der APOE Genotyp keinen differentiellen Einfluss auf die neuropsychologischen Leistungen, die Hirnvolumina und die arbeitsgedächtnis- und aufmerksamkeitsbezogene Hirnaktivität hatte.

Unsere Resultate deuten darauf hin, dass das APOE ε4 Allel einen vorteilhaften Einfluss auf die Gedächtnisfunktionen im jungen Erwachsenenalter ausübt. Dies manifestiert sich in den jungen ε4 Trägern als ein besseres episodisches Gedächtnis und als ein sparsamer Gebrauch gedächtnisbezogener neuronaler Ressourcen.

Die zweite Studie zielte darauf ab, frühe präklinische Merkmale der Alzheimer Demenz zu detektieren. Mutationen im PSEN1 Gen werden autosomal dominant an die Nachkommen weitergegeben, und führen zur familiären Form der Alzheimer Demenz (FAD). Diese ist, im Vergleich zur sporadischen AD, durch einen früheren Beginn und einen schnelleren Krankheitsverlauf charakterisiert. Wir untersuchten fünf nicht erkrankte Mitglieder einer Familie mit FAD. Ein junges Familienmitglied (20-jährig) und ein Familienmitglied mittleren Alters (45-jährig) trugen die C410Y Mutation im Presenilin1 Gen. In Familien mit dieser Mutation beginnt die Krankheit im Alter von etwa 48 Jahren.
Beide Mutationsträger zeigten selektiv schlechtere Gedächtnisleistungen in episodischen Gedächtnisaufgaben. Ausserdem wurde im jungen Mutationsträger während des episodischen Lernens und Erinnerns eine erhöhte Hirnaktivität in einem gedächtnisbezogenen Netzwerk gefunden, wenn dieser mit 21 gesunden, jungen Kontrollprobanden und mit Familienmitgliedern ohne Mutation verglichen wurde. Im Gegensatz dazu, zeigte die Mutationsträgerin im mittleren Alter während des episodischen Lernens und Erinnerns eine verminderte Aktivität in gedächtnisbezogenen Hirnstrukturen.
Die verstärkte Hirnaktivität im jungen Mutationsträger reflektiert mit hoher Wahrscheinlichkeit kompensatorische Mechanismen, welche Folge der zunehmenden, gedächtnisschädigenden AD Neuropathologie sind. Auf der anderen Seite reflektiert die reduzierte Hirnaktivität in der Mutationsträgerin mittleren Alters höchstwahrscheinlich eine substantielle neuronale Dysfunktion, welche auf die weiter fortgeschrittenen, neuropathologischen Ablagerungen zurückzuführen ist. Diese Daten zeigen, dass präklinische Gedächtnis Dysfunktionen, durch die Anwendung funktioneller Bildgebung und sensitiver Gedächtnistests, 30 Jahre vor dem klinischen Beginn der Krankheit erkannt werden können.

Summary

There's an ongoing debate about what extent nature and/or nurture influence human cognitive capacities like memory. Twin studies suggest a roughly 50% heritability estimate, indicating that naturally occurring genetic variations have an important impact on the interindividual variability in memory abilities. Genetic analyses, functional and structural magnetic resonance imaging, and quantitive neuropsychological methods were combined in this doctoral thesis to determine the influence of the apolipoprotein E (APOE) gene and the presenilin 1 (PSEN1) gene on normal memory performance, memory-related brain activity, and neuroanatomy.

In the first study, we sought to learn, whether the APOE genotype plays a role in normal memory function. The APOE gene has three allelic variants; ε2, ε3, and ε4. The ε4 allele is associated with an increased risk of the development of sporadic late onset Alzheimer's disease (AD), and detrimental effects of ε4 have been shown in middle-aged and elderly persons on cognition, memory-related brain activity, and brain glucose metabolism. As yet, an effect of ε4 on normal memory in absence of AD neuropathology is unknown.

We found better episodic memory retrieval in ε4 allele carriers, as compared to the ε2 and ε3 alleles, in a sample of 340 young, healthy persons. Using functional magnetic resonance imaging (fMRI) in a subset of 34 memory-matched subjects, we could show genetic effects on memory-related brain activity. Carriers of the ε4 allele decreased brain activity during the repeated learning of visual stimuli in a memory-related network and exhibited reduced retrieval-related activity. The ε2 and ε3 allele carriers, on the other hand, increased brain activity during repeated learning and exhibited enhanced retrieval-related activity. These genotype induced brain activity differences appeared although APOE isoforms had no differential effects on neuropsychological performance, brain volumes and brain activity related to working memory and attention.

These results suggest a beneficial effect of the APOE ε4 allele in young adulthood, emerging as better episodic memory and an economic use of memory-related neural resources.

The second study aimed at the preclinical detection of early markers for AD. Mutations on the PSEN1 gene are transmitted in an autosomal dominant manner and lead to familial Alzheimer's disease (FAD). FAD is characterized by an earlier onset and a faster disease progression compared to sporadic AD.

We examined five nondemented members of a family with FAD. A young (20 years) and a middle-aged (45 years) person carried the C410Y mutation on the PSEN1 gene. Disease onset in families bearing this mutation is around 48 years. Both mutation carriers showed selective memory impairments in tasks of episodic memory. Moreover, the young mutation carrier exhibited enhanced brain activity in a memory-related network during episodic learning and retrieval, as compared to 21 healthy control subjects and to family members without the mutation. In contrast, the middle-aged mutation carriers showed decreased activity in a memory-related network during episodic learning and retrieval.

The enhanced activity in the young mutation carrier might reflect compensatory mechanisms in response to accumulating, memory-affecting AD neuropathology. Conversely, reduced activity in the middle-aged mutation carrier might reflect substantial neural dysfunction in more advanced stages of AD-related neuropathological deposition.

Our data suggest, that by applying functional neuroimaging and sensitive memory tasks, preclinical memory dysfunction can be detected three decades before clinical onset of AD.

Introduction

Our memories of everyday life – of people, places and events – define who we are (Squire and Kandel, 1999). Life without memory is hardly imaginable, since our thoughts, goals and wishes are almost constantly dependent on memories of ourselfes, of our experiences and our environment. In a certain sense, our own personality is strongly connected to our memory. I would go even further and claim that our own personality is mediated to a large extent by our memories (although, it was not the aim of this doctoral thesis to prove that…). The most striking example for this notion comes from Alzheimer's Disease (AD) patients. Everybody who works or lives with AD patients can approve what drastic consequences the process of memory loss during the course of the disease has on the personality and the character of such a person. The devastating effects that memory disorders can have on normal life, was one of the major points why I got interested in studying memory. During my psychology study at the University of Zurich neuroimaging became more and more a valid and established method for investigating basic functions of human cognitive capacities and their underlying neural correlates, thereby, to some extent, bridging the gap between structure and function. Research on the brain literally exploded during that time, and this period was accordingly named 'Decade of the brain'. For my part, I was fascinated, that one could observe the brain at work. The logical consequence was a diploma thesis on the cognitive function of my primary interest – memory – investigated with the method that fascinated me the most – functional magnetic resonance imaging (fMRI). During my diploma work I started to gain extensive knowledge about memory and its neural correlates and learned a lot about the power of neuroimaging – and its pitfalls. After my psychology masters degree, I was ready to go a step further and deepen my knowledge. When I started the PhD program in cognitive neuroscience, the first studies were published that investigated genetic influences on normal memory function using neuroimaging techniques. I was attracted by the interdisciplinary nature of this research and decided to work on two projects combining genetics, neuroimaging techniques and quantitative neuropsychological methods.

There is an ongoing debate about the extent to which nature and nurture influence human cognition. About 20'000 genes are considered to play a role in the development, plasticity and maintenance of the central nervous system (CNS). 6 million single nucleotide polymorphisms (SNPs) are believed to be implicated in the genetic variability in worldwide populations, but it is likely that only a minority of the common variations is functional in nature, thus resulting in changes in the expression or the behavior of coded proteins. The variation in normal human

cognition is related to many factors. Twin studies suggest, that about 50% of the interindividual variability in memory ability is attributable to genetic factors (McClearn et al., 1997), thus individual single gene effects are expected to be small. This doctoral thesis is part of an attempt to identify genes that influence memory capacity, memory-related brain function and structure in health and in AD. One study focused on the influence of naturally occurring genetic variations on normal interindividual mnemonic capacities in young persons. The other study sought to determine preclinical signs of AD in carriers of an autosomal dominantly transmitted causative gene associated with a familial form of AD.

In the following theoretical part I will first give a short overview on the memory systems model, emphasizing on episodic and semantic memory and its neural correlates, because we mainly examined genetic influences on declarative memory. I will then introduce studies that reported polymorphisms in the brain derived neurotrophic factor (BDNF) gene, the serotonin (5-HT) 2a receptor encoding gene, and the prion protein (PRNP) gene which have been shown to influence normal human memory variability. Finally, I will review literature about the two genes that were examined in this doctoral thesis; the apolipoprotein E gene (APOE) and the presenilin 1 gene (PSEN1).

1. Genes and memory

1.1. Episodic memory and its neural correlates

Memory is divided into short-term and long term memory. In cognitive psychology short term memory is also termed 'working memory' and referred to as a limited capacity system allowing the temporary storage (up to one minute) and the manipulation of information necessary for such complex tasks as comprehension, learning and reasoning (Baddeley and Hitch, 1974). Long-term memory contents, on the other hand, are thought to be consolidated through protein synthesis at the synapse, leading to a definitive and permanent storage of information over time, hence enabling the recall of recent and remote events. The parcellation of long-term memory into distinct subsystems has a long history but became a topic of experimental inquiry only after the middle of the 20th century. In 1957 Scoville and Milner reported the case H.M., probably the most famous patient in memory research. H.M suffered from medication-resistent epileptic seizures. After the bilateral resection of the medial temporal lobes (MTL) the seizures vanished, but, as a consequence, the patient lost its ability to acquire new memories (anterograde amnesia) and the ability to consciously retrieve memories formed before the surgical intervention (retrograde amnesia). Nevertheless, short-term memory, intelligence, motor, perceptual, language and attentional abilities were preserved. This was the first report that made clear, that MTL structures, especially the hippocampus at its core, are of crucial importance for long-term memory (see Fig. 1 one for an illustration of MTL structures, including the hippocampus proper with fields CA1-CA4, dentate gyrus and subiculum, the rhinal cortices and the parahippocampal gyrus).

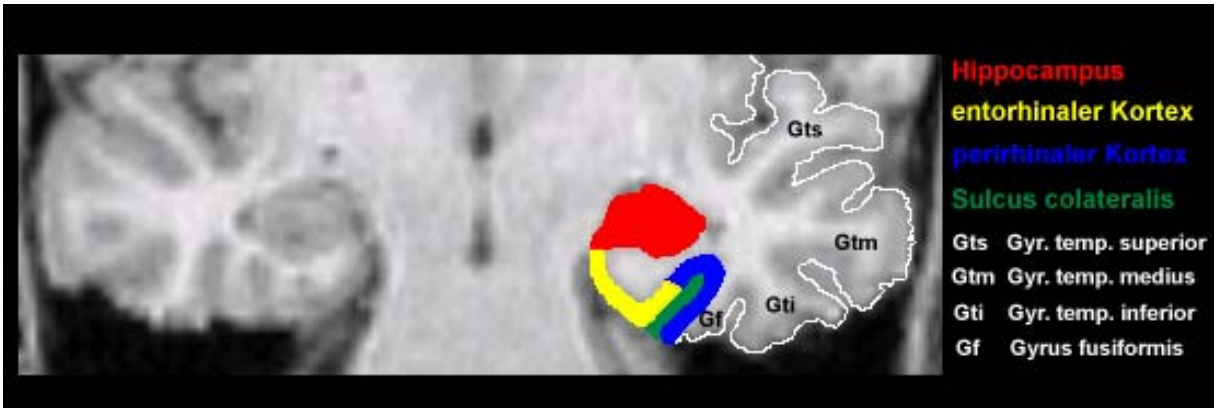

Fig. 1. Coronal view of the anatomical subdivisions of the MTL depicted on a structural T1-MRI scan of the author's brain.

Cohen and Squire (1980) observed an impaired ability to learn and remember declarative or data-based information in amnesic patients, while learning and remembering of procedural or rule-based information was preserved. This differentiation between the impairment for declarative memory (knowing what) and the preservation of non-declarative memory (knowing how) was the basis for a model of long-term memory systems, as proposed by Squire and Zola (1996). In this model, long-term memory is divided into declarative and non-declarative memory (Fig. 2).

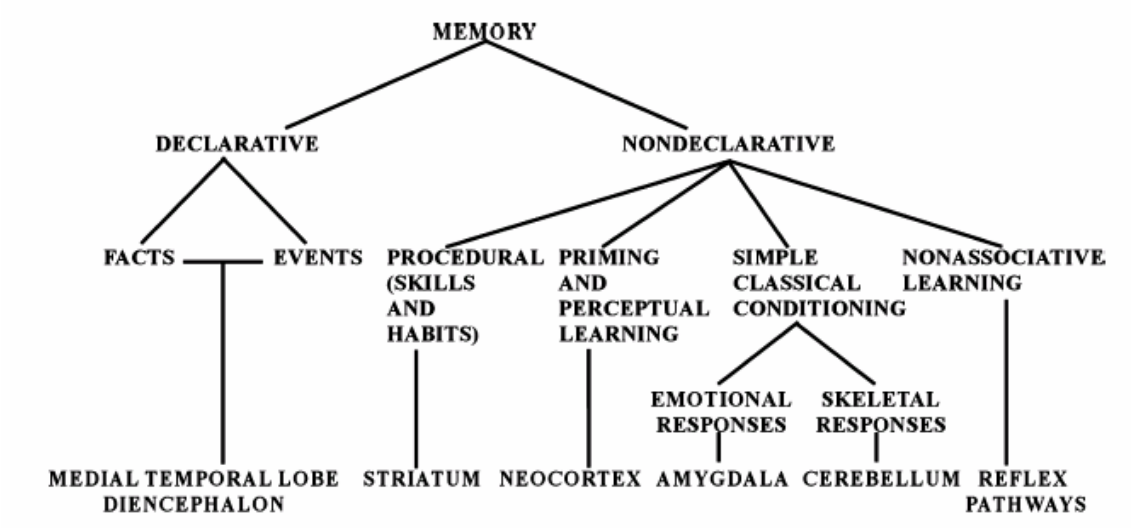

Fig. 2. A taxonomy of mammalian long-term memory systems. Listed are brain structures thought to be especially important for each form of declarative and nondeclarative memory (Squire and Zola, 1996).

Declarative memory is defined as the acquisition, retention, and retrieval of knowledge that can be consciously and intentionally recollected (Cohen and Squire, 1980). Declarative memory was further subdived by Tulving (1972) into memory for events (episodic memory) or facts (semantic memory). Tulving characterized episodic memory as the capacity to recollect where and when past events occurred. Because of their spatio-temporal connotation episodic memories are often referred to as autobiographical memories. Tulving and colleagues have recently refined their definition of episodic memory as the emphasizing qualities of the subjective consciousness and a sense of time in retrieving past experiences (Tulving, 2002; Tulving and Markowitsch, 1998). An example: If you think about yesterday evening, when you were riding with your bike along the river, you might consciously and vividly re-experience that episode. You might recall the time you were riding your bike, what the color of the river looked like or even what you were thinking during your little trip out in the nature. All these memory contents are of an episodic character because of their spatio-temporal context. Semantic memory contents, however, are not attributable to any specific

time or place. Typical contents of semantic memory would be general factual knowledge of the world, in our example this would be knowledge about bicycles, such as bikes have two wheels, two pedals, a bell to ring, etc.

Experimentally, declarative memory is accessed through explicit learning tasks, where participants are presented with stimuli that have to be retrieved after a determined delay.
There are three different forms of retrieval tests: a) free recall, where participants have to freely retrieve learned stimuli without any help or hint; b) cued recall, where participants are given a cue which elicits the retrieval of the stimulus, for example the first letter or syllable of previously presented words; c) recognition, where stimuli are presented along with one or more new distracter items and where participants then have to indicate the previously learned stimuli.

As mentioned above, studies with amnesic patients (Kartsounis et al., 1995; Rempel-Clower et al., 1996; Zola-Morgan et al., 1986) indicated that episodic declarative memory depends on the integrity of MTL and diencephalic structures, which interact with the neocortex. The hippocampal system is anatomically inter-connected with polymodal neocortical association areas; afferent input from the neocortex converges into the entorhinal and perirhinal cortex, is then projected to the hippocampal subdivisions and finally backpropagated to the neocortex through the parahippocampal gyrus (Amaral et al., 1987; Cohen and Eichenbaum, 1993; Insausti et al., 1987a; Insausti et al., 1987b; Squire and Zola, 1996; Van Hoesen and Pandya, 1975; Van Hoesen et al., 1975; Van Hoesen and Pandya, 1975). This anatomical feature allows the hippocampal system to interact with neocortical sites, providing the ability for the relational binding of information coming from different cortical sites (Cohen and Eichenbaum, 1993). Cortical areas that have been activated at the time of encoding can be reactivated by the hippocampal system, thereby accomplishing the retrieval of a whole memory.

Studies using quantitative methods to characterize memory loss in patients with more circumscribed lesions found that hippocampal damage, in particular, is typically associated with temporally-graded retrograde amnesia (Beatty et al., 1987; Rempel-Clower et al., 1996; Salmon et al., 1988; Squire et al., 1975). According to this evidence, the ‘consolidation hypothesis’, posits a time-limited role of the hippocampus in the storage and retrieval of new information, while permanent storage depends on a broadly distributed cortical network. The neural network model of consolidation suggests that MTL structures bind together multiple neocortical sites where memory representations are stored and that after repeated re-activation

the cortico-cortical connections become strong enough to support memory retrieval without the MTL (McClelland et al., 1995; Squire and Alvarez, 1995).

With the advent of neuroimaging techniques, the precise role of the hippocampal formation and surrounding MTL structures has further been refined. Activity in the hippocampal region has been correlated with the associative, recollective, and contextual aspects of declarative episodic memory (Cabeza and Nyberg, 2000; Cohen and Eichenbaum, 1993; Davachi et al., 2003; Henke et al., 1997; Henke et al., 1999b; Lepage et al., 2000; Mayes et al., 1998; Montaldi et al., 1998; Schacter and Buckner, 1998; Small et al., 2001; Sperling et al., 2001). However, the findings do not reveal a sharp distinction between the hippocampal region and the adjacent MTL structures. Recent findings implicated both the hippocampal region and the parahippocampal cortex in recollective memory and in the encoding and retrieval of associations (Davachi et al., 2003; Duzel et al., 2003; Eldridge et al., 2000; Henke et al., 1997; Henke et al., 1999b; Kirwan and Stark, 2004; Ranganath et al., 2004; Small et al., 2001; Sperling et al., 2001; Sperling et al., 2003; Stark and Squire, 2001; Yonelinas et al., 2001).

The neural topology of semantic declarative memory is less clear. Studies in patients suffering from semantic dementia have identified neocortical areas of the temporal lobe, such as the left inferior frontal gyrus and the lateral temporal gyri as the underlying neural substrates (Cabeza and Nyberg, 2000; Kopelman, 2002; Martin and Chao, 2001; Pilgrim et al., 2002; Wagner et al., 2001). Patients with semantic dementia are struck by focal atrophy in the temporal neocortex, while the MTL is relatively spared. As a consequence, they are strongly impaired in the retrieval of general facts of the world but show almost normal performance in tasks of episodic memory. It is still a matter of debate, whether the hippocampus is involved in the retrieval and the acquisition of semantic memory (Squire and Zola, 1998), the acquisition only (Cipolotti et al., 2001) or none of both (Vargha-Khadem et al., 1997).

In contrast to declarative memory, nondeclarative memory is divided into heterogeneous subforms, such as procedural memory (skills and habits), priming and perceptual learning, simple classical conditioning and nonassociative learning. These kinds of memory are not dependent on conscious awareness for the learning episode or the learning material and are mostly preserved in amnesic patients with MTL structure damage. Nondeclarative memory is subserved by different extra-MTL structures such as the neocortex, cerebellum, striatum,

amygdala and reflex pathways (for reviews please see (Gabrieli, 1998; Squire, 1992; Squire et al., 1993).

1.2. The brain-derived neurotrophic factor (BDNF) gene, the serotonin (5-HT) 2a receptor encoding gene and the prion protein gene (PRNP)

As yet, three genetic polymorphisms have been identified that play a role in the normal variabilty of human memory capacity. De Quervain and colleagues (2003) investigated the effects of a frequent polymorphism (H452Y) of the gene encoding the serotonin (5-hydroxytryptamine or 5-HT) 2a receptor on human memory. This polymorphism predicts an amminoacid substitution (His to Tyr) at residue 452. Serotonin has been implicated in learning and memory. Its receptor subtypes, including the 5-HT2a receptor, are localized on 'cognitive' pathways, with the hippocampus and frontal cortex as the main target structures (Buhot, 1997). The authors examined 349 subjects, split into two independent populations of either university students (academics) or age-matched employees/trainees without an university degree (non-academics). Subjects engaged in a word learning/retrieval task that was conducted on two consecutive days. On the first day, subjects learned six sets of semantically unrelated nouns (five nouns per set), presented at a rate of one word per second, for immediate recall. An unexpected delayed free recall of the learned words was conducted after 5 minutes and after 24 hours. In both, the academic and the non-academic group, His/Tyr subjects showed 21% poorer memory performance in the 5 minutes delayed free recall. Furthermore, after splitting the whole sample into good and poor learners, a higher proportion of His/Tyr subjects were found in the poor learners group. These results suggest that the (H452Y) polymorphism of the 5-HT2a receptor encoding gene negatively affects protein-synthesis independent memory recall after 5 minutes but not after 24 hours or during immediate recall. However, although the study identified one of the presumably many genes involved in normal human memory capacity, it was not able, because of its associative nature, to uncover neural mechanisms underlying the poorer performance of polymorphism carriers.

Papassotiropoulos et al. (2005) investigated the effects of the common Val129Met polymorphism of the prion protein gene (PRNP) on long-term memory in 354 healthy young humans. Long-term memory depends on protein synthesis and on molecular long-term changes at the neuronal synapse (Kandel, 2001). It has been shown in yeast and *Aplysia*[1] that

[1] Aplysia: a marine snail in which substantial research on the molecular basis of short and long-term memory has been conducted, primarily by Nobel Prize winner Eric R. Kandel and co-workers.

a prion-like protein switch might help to maintain long-term synaptic changes (Si et al., 2003a, 2003b), suggesting that genes encoding prion-like properties could be associated with human long-term memory. The prion protein Prp is encoded by the polymorphic PRNP gene in humans. The common methionine/valine polymorphism at codon 129 (M129V) modulates the folding behaviour of the protein and controls susceptibility to prion diseases (Petchanikow et al., 2001; Tahiri-Alaoui et al., 2004; Palmer et al., 1991). Papassotiropoulos et al. (2005) found that the three investigated single nucleotide polymorphisms[2] (SNPs) (i.e. the 129^{MM}, the 129^{MV}, and the 129^{VV} genotype) in the genomic region of PRNP were significantly associated with long-term memory but not with short-term memory. Moreover, after excluding confounding factors such as adjacent genomic loci, population structure and sampling bias the authors found that subjects homozygous or heterozygous for the 129^{M} variant exhibited 17% better memory performance in the 24 hours recall of previously learned words. These results suggest that the human prion protein in genetically associated with long-term memory. However, like de Quervain et al. (2005), this study could not deliver substantial information on the neural mechanisms underlying better memory in the met allele carriers.

A third gene to play a role in human memory is the brain derived neurotrophic factor (BDNF) encoding gene. BDNF is expressed in the brain, most strongly in the hippocampus (Murer et al., 2001). It has been implicated in both early phase long-term potentiation (E-LTP) (Bliss and Collingridge, 1993), which involves rapid increases in intracellular calcium concentrations and subsequent activation of protein kinases, and late phase long-term potentation (L-LTP) (Kandel, 2001), which is characterized by the recruiting of the cAMP and CREB signalling pathways to direct protein synthesis-dependent changes in structure and function (Lu and Gottschalk, 2000; Poo, 2001). Moreover BDNF is involved in the regulation of cell survival, proliferation, and synaptic growth in the developing central nervous system (CNS). Val66met is the only reported common polymorphism that has been identified in the BDNF gene. It is a SNP at nucleotide 196 (G/A) producing an aminoacid substitution (valine to methionine) at codon 66. Egan and colleagues (2003) found that rat hippocampal neurons transfected with the met allele[3] exhibit abnormal intracellular trafficking and regulated secretion of BDNF in comparison to those transfected with the val allele. The same study

[2] SNP (single nucleotide polymorphism): a variant in a single nucleotide at a specific location in the genomic sequence that may or may not have functional consequences for the protein, depending on the type of substitution and the context. A non-functional SNP (i.e. the SNP is silent or in a non-coding area of the gene) may still be in a monitoring relation with a functional mutation (e.g. if it is physically close on the genomic sequence).

[3] Allele: a variant of a gene

further established an association of the met allele with deficits in episodic memory in healthy human subjects and showed that the met allele is linked with diminished levels of hippocampal N-acetyl aspartate, a putative marker of neuronal integrity and synaptic abundance. To tackle the contribution of the BDNF val66met polymorphism on human memory and memory-related hippocampal activity, Hariri and colleagues (2003) studied 28 healthy volunteers with fMRI while they encoded and subsequently retrieved complex, novel scenes. This simple declarative memory task is known to be dependent on the hippocampal formation (Gabrieli, 1998). 14 subjects carried the Val/Val genotype[4], the variant associated with normal intracellular trafficking of BDNF and better episodic memory, while 12 subjects carried the val/met and two the met/met genotype. Importantly, the genotype groups were matched for gender, age, mean intelligence quotient and the apolipoprotein E ε4 allele. The apolipoprotein E ε4 allele[5] has been associated with a higher risk for the development of late onset sporadic Alzheimer's Disease (Corder et al., 1993) and was found to have an impact on memory-related brain activity in healthy elderly subjects (Bondi et al., 2005; Bookheimer et al., 2000). The comparison of both groups revealed greater memory-related hippocampal activity during encoding and retrieval in subjects homozygous for the BDNF Val allele. However, BDNF genotype did not alter activity within a distributed network (e.g. inferotemporal, parietal and frontal areas) subserving general visuospatial information processing, hence strengthening the specificity of this BDNF effect on hippocampal activity. In the memory tasks, Val homozygotes were more accurate at recognizing both 'new' and 'old' scenes during retrieval, although encoding accuracy was comparable between groups. Moreover, using a modified hierarchical stepwise regression analysis, the authors showed that ~25% percent of the total variation in recognition memory was explained by an interaction term between the BDNF val66met genotype and the mean left hippocampal activity during encoding.

A recent study of the same group (Pezawas et al., 2004) showed that the val66met variation in the BDNF gene not only impacts on memory performance and memory-related hippocampal acitivty, but also on hippocampal and prefrontal cortex morphology, brain areas related to learning and memory. The authors analyzed high-resolution magnetic resonance images of 111 normal healthy volunteers with optimized voxel-based morphometry (VBM), an automated morphological imaging technique (Ashburner and Friston, 2000). Bilateral

[4] Genotype: allelic combination at a locus (expressed as homozygote aa, homozygote AA, or heterozygote Aa)

[5] Please refer to chapter 1.3. of this doctoral thesis for a comprehensive overview of the apolipoprotein E gene and its implication in Alzheimer's Disease, declarative memory, memory-related neural activity, and brain morphology.

reductions of hippocampal gray matter volumes and volume reductions in the dorsolateral prefrontal cortex were found in met carriers compared with Val homozygous carriers. According to the authors, these data suggest, that reduced memory functions of met carriers might, at least in part, be explained through changes of synaptic and cellular plasticity and not exclusively by an effect of rapid changes of neural transmission during memorization. They add, that the mechanism of this structural effect presumably relates to abnormal regulated secretion of met-BDNF alleles, which alters activity-dependent processes of cortical development and plasticity.

In this section I reviewed studies that identified genes that are involved in normal human memory variability. So far, three polymorphisms have been detected to play a key role; the val66met polymorphism in the BDNF gene, the H452Y polymorphism in the 5-HT2a receptor encoding gene, and the Met129Val polymorphism in the prion gene. While the H452Y polymorphism of the 5-HT2a receptor encoding gene was associated with poorer memory performance in young and healthy subjects, the common Met129Val polymorphism in the PRNP gene was related to better long-term memory performance. Last but not least, the val66met polymorphism in the BDNF gene was confirmed to have an impact on hippocampal cognition, neurophysiology, neurochemistry and morphology. In the following two sections, I will review literature about the genes of interest in this doctoral thesis; the APOE gene and the PSEN1 gene. The implication of both the APOE gene and the PSEN1 gene on normal human memory variability are still unknown, therefore a focus will be laid on studies that examined effects of these genes on memory in light of a preclinical detection of markers for AD.

1.3. The apolipoprotein E (APOE) gene

Apolipoprotein E (apoE) has emerged as an important molecule in several biological processes. This plasma glycoprotein is involved in cholesterol metabolism, immunoregulation and cognition (Mahley and Rall, Jr., 2000). A primary metabolic role for apoE is to transport and deliver lipids from one tissue or cell type to another (Mahley, 1988; Mahley and Huang, 1999; Mahley and Ji, 1999). The three major isoforms, apoE4, apoE3, and apoE2 can be distinguished from one another only by single amino acid substitutions. These changes have profound functional consequences at both the cellular and molecular levels. Isoform specific effects on the normal variation of plasma lipids have been described in population studies.

These studies have reported, that apoE3 contributes little to variation, whereas apoE2 and apoE4 have definite impacts on lipid and lipoprotein levels (Utermann, 1985).
ApoE is a polymorphic protein arising from three alleles at a single locus. In humans the Apolipoprotein E gene is located on chromosome 19. It is synthesized and secreted primarily by the liver, brain, skin, and tissue macrophages throughout the body (Mahley, 1988). It possesses two tightly linked coding sequence polymorphisms, which give rise to three allelic variants (ε2, ε3, ε4). Thus, six phenotypes are possible and have already been detected in human subjects: three homozygous phenotypes (ε2/ε2, ε3/ε3, ε4/ε4) and three heterozygous phenotypes (ε2/ε3, ε2/ε4, ε3/ε4). Population studies yielded valuable information about APOE allele frequencies: the most common variant, ε3, is present in approximately 75% in Caucasians, while ε4 and ε2 are present in only 15% and 10%, respectively (St George-Hyslop, 2000). As a result, the frequencies of the six APOE phenotypes are approximately: 55% ε3/ε3, 25% ε3/ε4, 15% ε2/ε3, with the other phenotypes ε2/ε2, ε4/ε4, ε2/ε4 making up the remaining 1-2 % (Mahley and Rall, Jr., 2000).

The APOE ε4 allele (APOE4) has an extensive record of deleterious effects on several biological processes. It has been associated with increased heart disease risk (Davignon et al., 1988; Menzel et al., 1983; Utermann et al., 1984) and coronary artery disease (Davignon et al., 1987; Wilson et al., 1994). The most prominent role of APOE4, however, is it's association with an increased risk for developing sporadic late onset Alzheimer's disease (Corder et al., 1993; Polvikoski et al., 1995; Roses et al., 1994; Saunders et al., 1993; Strittmatter et al., 1993a). Carriers of at least one ε4 allele have a threefold increased risk for the development of AD (Saunders et al., 1993) with a younger age at the onset of dementia (Corder et al., 1993; Roses et al., 1994). APOE4 is further associated with the hallmarks of AD - amyloid plaque deposition and neurofibrillary tangle formation (Bennett et al., 2003; Tiraboschi et al., 2004), with a faster rate of ß-amyloid protein deposition (Roses, 1995; Schmechel et al., 1993), and with decreased longevity (Cauley et al., 1993; Davignon et al., 1987; Schachter et al., 1994). In contrast, the possession of an ε2 allele appears to confer a protective effect against AD (Corder et al., 1994). It has been further associated with an older age at the onset of dementia (Roses et al., 1994), with a reduced decline in episodic memory in older persons (Wilson et al., 2002), slower rates of ß-amyloid protein and neurofibrillary tangle deposition (Polvikoski et al., 1995; Schmechel et al., 1993), a decreased risk of atherosclerosis (Davignon et al., 1988; Wilson et al., 1994), and increased longevity (Cauley et al., 1993; Davignon et al., 1987; Schachter et al., 1994).

Detrimental effects of APOE4 on memory (Albert et al., 2001; Backman et al., 2001; Baxter et al., 2003; Bondi et al., 1995; Bondi et al., 1999; Chen et al., 2001; Fuld et al., 1990; Grober and Kawas, 1997; Hyman et al., 1996), executive functions (Rosen et al., 2005), and visual attention (Greenwood et al., 2000) have been found in several studies with nondemented middle-aged and elderly subjects. Often the decline in episodic memory is evident some years prior to the development of dementia and has been shown to predict the subsequent development of AD. For example Bondi et al. (1999) found poorer mean performances on delayed recall in 43 nondemented older APOE4 carriers when compared neuropsychologically to participants without a copy of the ε4 allele. At baseline the groups did not differ on age, gender, education, or global cognitive status. Significantly more APOE4 participants developed probable or questionable AD, compared with non-APOE4 participants, suggesting that group differences resulted from a preponderance of preclinical AD cases within the ε4 group and not from a direct influence of APOE genotype on cognition. In a recent longitudinal study Caselli and colleagues (2004) tested 45 APOE ε4/ε4 carriers, 42 APOE ε3/ε4 carriers and 93 APOE ε4 noncarriers with a mean age of 60 years. APOE ε4 carriers among 50 to 59 years exhibited poorer memory performance, as compared to ε4 noncarriers, in multiple measures of verbal memory over a mean period of 33 months. No differences were found in the domains of language, spatial skills, or executive functions.

Detrimental effects of APOE4 are, however, not restricted to episodic memory, but do encompass other cognitive functions as well. Rosen et al. (2005) tested twenty ε4 carriers and twenty ε4 noncarriers on a category fluency task. Subjects were tape recorded while saying animal names for ten minutes. The ε4 carriers generated significantly fewer names and clusters of animals, and took significantly longer to access clusters when compared to the ε4 noncarriers. The authors attributed the APOE4 carriers' deficits in executive functions to a reduced attentional capacity. Indeed Greenwood et al. (2000) showed that specific components of visual attention are affected by the APOE genotype. They found that middle-aged nondemented APOE4 carriers exhibited deficits when visual attention was spatially directed by cues in tasks of visual discrimination and visual search, in comparison to APOE4 noncarriers. Consistent with the aforementioned findings linking APOE and cognition are results from a study which showed that the absence of APOE4 is associated with higher levels of cognitive functioning in very old (75-98 years) adults (Riley et al., 2000).

The association of APOE4 with the hallmarks of AD - amyloid plaque deposition and neurofibrillary tangle formation (Bennett et al., 2003; Tiraboschi et al., 2004) – suggests that

APOE4-related cognitive deterioration is mediated by these neuropathological events. Support for this notion comes from studies with structural and functional neuroimaging. Amyloid deposition and neurofibrillary tangle formation lead to neuronal loss and synaptic degradation that is manifested as brain morphological loss in AD, starting in MTL structures and spreading throughout the neocortex in later stages of the disease (Braak and Braak, 1996; Delacourte et al., 1999). Hippocampal volume loss is consistently found in clinically diagnosed AD patients (Convit et al., 1997; Kesslak et al., 1991; Killiany et al., 1993). Hippocampal atrophy in older persons with mild cognitive impairment (MCI) is associated with subsequent development of clinical AD (de Leon et al., 1993; Killiany et al., 2000). Similar but less pronounced changes in hippocampal morphology occur in healthy adults with APOE4. A study with ten pairs of cognitively normal twins (mean age = 62.5 years) found that individuals carrying an APOE ε4 allele had smaller hippocampi than those lacking an ε4 allele, despite comparable performance levels on standardized neuropsychological tests (Plassman et al., 1997). Reiman et al. (1998) compared hippocampal volumes of 11 cognitively normal homozygous APOE4 carriers (50 to 62 years of age) to 22 persons without APOE4. The APOE4 homozygotes showed nonsignificant trends for smaller left and right hippocampal volumes. Overall, small hippocampal volumes were associated with reduced performance on a long-term memory test. A recent study (Lemaitre et al., 2005) using both voxel-based morphometry and region of interest analysis for hippcampal volume estimation studied a cohort of 750 healthy elderly volunteers (age range 63-75 years). Significant decrease of grey matter in ε4 homozygous subjects (n=12), as compared both to ε4 heterozygous subjects (n = 175) and to noncarriers (n = 563) was found bilaterally in the MTL, including the hippocampus and extending over the superior temporal gyrus. However, no significant difference was observed between ε4 heterozygous subjects and noncarriers at the level of the MTL. Follow up of the cohort cognitive performances over 4 years revealed an increased relative risk of the homozygous ε4 carriers for cognitive impairment. These results do not argue against a gene-dose dependent effect of APOE on brain morphology since a present ε4 effect on hippocampal volumes was indeed present in heterozygotes, but was small enough to have been overwhelmed by the much greater effect of intersubject anatomical variability that increases with age (Jack, Jr. et al., 1998).

Positron emission tomography (PET) studies of clinically diagnosed AD patients have shown reduced cerebral metabolism and blood flow in temporal and parietal cortices (Alexander et al., 1997; Haxby et al., 1985; Ibanez et al., 1998; Rossor et al., 1996). Similar metabolic

changes measured with PET have been reported in APOE4 carriers (de Leon et al., 2001; Reiman et al., 2002a; Reiman et al., 2002b; Reiman et al., 2004; Reiman et al., 2005; Reiman et al., 1996; Scarmeas et al., 2004; Small et al., 2000; Small et al., 1995). Small et al. (1995) examined middle-aged persons with MCI and with a positive family history of AD. APOE4 carriers showed a lower overall level of parietal metabolism and greater hemispheric asymmetry. These findings were confirmed in a follow-up study with another sample of participants with MCI (Small et al., 2000). Here, a single copy of the APOE ε4 allele was associated with lowered inferior parietal, lateral temporal, and posterior cingulated metabolism, which predicted cognitive decline after 2 years of longitudinal follow-up. A study with cognitively normal individuals found that APOE4 homozygotes showed reduced rates of glucose metabolism in parietal, temporal, prefrontal, and posterior cingulated regions (Reiman et al., 1996). A recent study of the same group examined 160 cognitively normal subjects 47-68 years of age with PET. The sample included 36 APOE4 homozygotes, 46 heterozygotes, and 78 APOE4 noncarriers who were individually matched for their age, gender, and educational level. The authors found that ε4 gene dose was correlated with lower cerebral metabolic rate for glucose in regions of the precuneus, the posterior cingulated, parietotemporal, and frontal cortex.

Two studies using functional imaging have reported altered patterns of brain activation in APOE4 carriers (Bondi et al., 2005; Bookheimer et al., 2000). Bookheimer et al. (2000) studied 30 subjects (aged 47 to 82 years) who were cognitively healthy, and of whom 16 were APOE4 carriers and 14 were homozygous APOE3 carriers. Mean age and level of education were matched between both groups. The blood oxygenation level dependent (BOLD) fMRI-signal was measured, while subjects memorized and recalled unrelated pairs of words and during a resting baseline condition. Interestingly, both the magnitude and the extent of brain activation during memory-activation tasks in regions affected by AD, including the left hippocampal, parietal, and prefrontal regions were greater among the carriers of the ε4 allele than among carriers of APOE3. During recall of the learned material, APOE4 carriers showed a greater average increase in signal intensity in the hippocampal region and greater mean number of activated regions throughout the brain. After two years 8 ε4 carriers and 6 ε3 carriers were reassessed. Significant activation in the left hemisphere at baseline was significantly correlated with the degree of longitudinal verbal memory decline. The authors argue that the increased level and volume of activation in the APOE4 group may effectively serve a compensatory role, wherein subjects use additional cognitive resources to bring memory-related performance to a normal level. Indeed, increased activation in prefrontal

cortex is also seen in AD patients and could be indicative of a compensatory response (Grady et al., 1993; Grady and Parasuraman, 1995). The same line of argumentation is followed by a recent study. Using fMRI, Bondi et al. (2005) examined twenty nondemented participants with normal episodic memory function during a picture-encoding task. Nondemented older adults with APOE4 (n = 10) showed greater magnitude and extent of BOLD brain response in multiple regions (e.g. precuneus, frontal, temporal, and cingulated gyri) during learning of new pictures relative to the matched APOE3 carriers. Again, the increased activity in APOE4 carriers was explained with the '*Compensatory Hypothesis*'. The authors hypothesize that after an initial decline in memory proficiency following damage to MTL structures, patients in the preclinical stage of AD are able to effectively recruit compensatory brain resources (e.g. frontal and temporal cortical regions important for executive functions and semantic memory) to halt or slow further memory decline for a period of time.

These neuroimaging studies have yielded generally consistent results. However, all studies were performed in middle-aged to elderly subjecs (50-65 years), which on one hand increases confidence that the findings are not confounded with age-related changes. On the other hand, these studies might only have mapped APOE4 effects through the progressing neuropathological changes in preclinical AD cases. Valuable insights into a distinct APOE genotype mediated effect on normal neurophsyiologic functioning of memory can only be achieved in healthy normal subjects that are, with great probability, devoid of any such neuropathological changes.

Interestingly, detrimental effects of APOE4 are almost only observed in middle-aged to older human subjects and in aged animals. APOE4 has been also experimentally related to impaired hippocampal plasticity (Levi et al., 2003) and lower density of dendritic spines (Ji et al., 2003) in aged transgenic mice.

Advantageous effects of APOE4 on the other hand, have almost exclusively been reported in utero, in childhood, or in young adulthood. Zetterberg et al. (2002) investigated the role of APOE polymorphisms in embryonic development and analyzed the APOE genotypes of 81 spontaneously aborted embryos and 110 adult controls. The ε4 allele was significantly less frequent in the spontaneous abortion group than in the control group, while the frequency of ε3 was significantly increased, suggesting that APOE4 might have protective effects during embryogenesis. Ravaja et al. (1997) measured heart rate (HR), finger blood volume, and skin conductance level during experimentally induced stress in 28 healthy 16-year-old boys. They found that APOE4 noncarriers showed significantly greater HR reactivity

and significantly greater task levels of HR and HR variability during mental stress, suggesting that APOE4 is associated with less cardiovascular responsivity to mental stress in adolescent boys. Beneficial effects of APOE4 were also found in human cognitive functions. Hubacek et al. (2001) found an association of APOE4 with higher education. In the group with higher education 24.4% had the ε4 allele, while only 7.3% carried the ε2 allele. An inverse relationship was found in subjects that left school at age 15 – 8.3% had the ε4 allele and 13.9% had the ε2 allele. Also, eighty-seven percent of the subjects with APOE4 reached higher education, while this was the case only for 54.5% of APOE2 carriers. This indicates a possibility for a role of APOE polymorphisms in intelligence and the ability to learn. As a matter of fact, Yu et al. (2000) found modest performance intelligence quotient increases in young ε4 allele carrier females.

While the detrimental effects of APOE4 seem to be tightly coupled to the neuropathology of AD (Kounnas et al., 1995; LaDu et al., 1997; LaDu et al., 1994; Ma et al., 1994; Miyata and Smith, 1996; Sanan et al., 1994; Strittmatter et al., 1993b; Wisniewski et al., 1994; Yankner et al., 1990), the beneficial effects might be best explained through an evolutionary line of argumentation (Finch and Sapolsky, 1999; Mahley and Rall, Jr., 2000). It has been suggested that, even though ε3 is the most common allele in humans, ε4 may be the ancestral allele (Hanlon and Rubinsztein, 1995; Mahley, 1988). One might expect, that both ε2 and ε4 should confer some selective advantages that have allowed them to persist in the human population. In support for the view that ε4 might be the ancestral allele is the fact, that most animals have E4-like apoE, and especially significant is that all great apes, the closest living ancestors of humans, have the ε4-like allele and do not display multiple isotypes (Hanlon and Rubinsztein, 1995). A lot of evidence so far suggests that ε4 is a deleterious allele. However, the question remains why it persisted at such a high frequency. One possible answer is delivered by Finch and Sapolsky (1999). They hypothesized that ε4 could have survived because of its advantageous effects early in life, when reproductive fitness is at its peak, but having a disadvantageous role later in life.

1.4. The presenilin 1 (PSEN1) gene

The presenilins are evolutionary conserved transmembrane proteins that regulate cleavage of certain other proteins in their transmembrane domains. The proteins encoded by the presenilin genes are 467 (PS1) and 448 (PS2) amino-acids long, and there is strong sequence homology

between the two (Czech et al., 2000). Presenilin homologues are found also in animals and plants, and a functional conservation of presenilins has been shown between humans and distantly related species (e.g. the nematode *C. elegans*) (Baumeister et al., 1997). The physiological functions of presenilins have been studied extensively in transgenic mouse models. PS1 has a specific role in neural development by acting on the notch[6] signalling pathway. Lack of PS1 results in an embryonic lethal phenotype, which is reminiscent to the phenotype caused by a notch knock out (Shen et al., 1997; Wong et al., 1997). Similar to the notch knock out, lack of PS1 expression results in abnormal pattering of the axial skeleton and spinal ganglia. Besides this, presenilins were also implicated in synaptic plasticity, learning and memory, and neuronal survival. A recent study (Saura et al., 2004) examined conditional double knock out mice lacking both presenilins in the postnatal forebrain and found impairments in hippocampal memory and synaptic plasticity. These deficits were associated with specific reductions in NMDA[7] receptors and αCaMKII[8]. In contrast, a higher degree of LTP induction mediated by the expression of variants in the presenilin 1 gene (PSEN1) has been shown by other groups in mice expressing additional PSEN1 mutations (Barrow et al., 2000; Pybus et al., 2003). Thus, PS1 may specifically regulate cellular components necessary for LTP induction, and mutations in PSEN1 disrupt the normal cascade of events, leading to alterations in intercellular communication.

The most prominent role of the presenilins, however, is their implication in AD pathogenesis. Early onset familial Alzheimer's Disease (FAD) is transmitted in an autosomal dominant manner and accounts for ~10% of all AD cases (St George-Hyslop, 2000). Mutations in three genes have been linked to FAD: The amyloid precursor protein gene (APP) on chromosome 21 (Goate et al., 1991), the presenilin 1 gene (PSEN1) on chromosome 14 (Alzheimer's Disease Collaborative Group, 1995; Sherrington et al., 1995) and the presenilin 2 gene (PSEN2) on chromosome 1 (Levy-Lahad et al., 1995; Rogaev et al., 1995). PSEN1 is expressed throughout the brain, interestingly at high levels in cerebral regions that are affected in AD (i.e. hippocampus, cerebral cortex and amygdala) and in peripheral tissues. PSEN2 is expressed only at low levels in the brain, except in the corpus callosum, but highly expressed in some peripheral tissues (Rogaev et al., 1997). Low PSEN2 levels in the brain are compensated by a higher activity of PSEN1. This might explain, why PSEN2 muations are infrequent and incompletely penetrant compared with the fully penetrant PSEN1 mutations (Bird et al., 1996; Sherrington et al., 1996).

[6] Notch is expressed in neural cells and is involved in the determination of neuronal fate during development.
[7] N-Methyl-D-Aspartate receptors are ionotropic glutamate receptors that are involved in long-term potentiation.
[8] Calmodulin dependent kinase II

As yet, more than 100 missense[9] mutations of the PSEN1 gene are known with varying ages of onset of AD ranging from 25 years to 64 years (Rogaeva, 2002; Tandon et al., 2000). These pathogenic mutations modify amyloid precursor protein (APP) processing thereby leading to an enhanced Aß42 secretion (Scheuner et al., 1996). AD patients carrying PSEN1 or PSEN2 mutations have significant increases of plasma Aß42 levels together with massive deposition of Aß42 in the brain (Iwatsubo, 1998; Lemere et al., 1996). The deposition of Aß42 is one of the earliest visible neuropathological features in AD. In FAD patients with PSEN1 muations, the deposition of Aß42 precedes overt neuronal loss (Lippa et al., 1998) or the appearance of neurofibrillary tangle formation, the other neuropathological hallmark of the disease (Braak and Braak, 1996).

A variety of studies investigated pedigrees with different mutations on the PSEN1 gene and described the clinicopathological features of FAD (Alzheimer's Disease Collaborative Group, 1995; Fox et al., 1997; Haltia et al., 1994; Heckmann et al., 2004; Janssen et al., 2000; Jimenez-Escrig et al., 2004; Rosselli et al., 2000). Neuropathologic changes in FAD are similar to changes in sporadic cases of AD, however, familial cases show initial clinical symptoms at an earlier age (Bird et al., 1989) and have a faster disease progression (Farlow et al., 1994) along with more abundant tissue histopathology (Gomez-Isla et al., 1997; Lippa et al., 2000; Sakamoto et al., 2002). Similar to sporadic cases of AD first neuropsychological symptoms in FAD are early deficits in verbal and figural delayed recall and progressive loss of language abilities (Alzheimer's Disease Collaborative Group, 1995; Lopera et al., 1997). Additionally executive functions can be affected, consistent with frontal lobe degeneration (Raux et al., 2000). A more rapid decline and early symptoms of aphasia and apraxia have been more commonly described in FAD than in sporadic AD (Godbolt et al., 2004; Lampe et al., 1994; Martin et al., 1991). However, certain PSEN1 mutations have been associated with atypical phenotypes including spastic paraparesis (Assini et al., 2003; Kwok et al., 1997; O'Riordan et al., 2002), speech production deficits (Kennedy et al., 1995), language impairment, and frontal behavioural disturbances (Jimenez-Escrig et al., 2004). Few studies have analyzed the longitudinal cognitive changes in FAD. Rosselli et al. (2000), to name one of the few, examined 12 patients with FAD (mean age = 49.6 years), 10 patients with sporadic AD (mean age = 71.4), and 15 matched normal controls (mean age = 45) with two neuropsychological instruments: 1) The Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) neuropsychological test battery, and 2) additional tests of abstraction and

[9] Missense mutation: variation in the protein coding sequence which may alter the function of the protein.

constructional abilities. Tests were administered three times over a period of 18 months. At the first examination, FAD patients were significantly impaired on all measures, except for reading of words. While the performance of the normal controls remained almost unchanged over the 18 months, the patients with FAD displayed a decline in verbal memory, language, constructional and abstraction test. As expected, patients with sporadic AD demonstrated a similar pattern of cognitive deterioration, however, decline was faster in FAD than in sporadic cases.

Persons in whom the development of AD can be reliably predicted provide a unique opportunity for the detection of early markers and the characterization of early stages of the disease. Although there have been made advances in the understanding of imaging (Masdeu et al., 2005) and biochemical biomarkers (Small, 2002) for AD, neuropsychological testing remains the milestone for diagnosing and characterizing the cognitive losses that occur early in dementia. In a recent study, Ringman et al. (2005) performed neuropsychological tests on 51 nondemented at-risk members of 10 mexican families with two distinct PSEN1 mutations. Test scores were compared between the 30 mutation carriers and the 21 noncarriers. After dividing the subjects into age tertiles, mutation carriers showed lower visuospatial and executive function/working memory but not verbal memory or language composite scores. However, differences between groups were only found in the oldest tertile, indicating that early neuropsychological markers for AD were not detectable decades before the onset of FAD. In another preclinical study, Fox et al. (1998) longitudinally assessed asymptomatic individuals at risk for FAD. 63 subjects were examined over a 6-year period on current intelligence, verbal and visual memory, naming, perception, arithmetic, spelling, psychomotor speed and attention. 10 subjects developed symptoms of episodic memory loss and subsequently progressed to fulfil criteria for possible or probable AD. Moreover, these subjects already had significantly lower verbal memory and performance scores at their first assessment, when they were presumably unaffected, and subsequently showed progressive decline in multiple cognitive domains. The mean time from first assessment to the appearance of symptoms was 2.6 years. Cognitive deficits were accompanied by diffuse cerebral and medial temporal lobe atrophy only once subjects were clinically affected. These findings suggest, that cognitive decline in FAD is measurable by means of neuropsychology several years before clinical onset, and that verbal memory deficits precede more wide spread deterioration.

By the time first clinical symptoms in AD or even MCI are detectable, a 15 to 30 years long period of continuous amyloid plaque deposition and neurofibrillary tangle formation has already occurred (Davies et al., 1988; Rumble et al., 1989), and neuronal loss has taken place to some extent (Gomez-Isla et al., 1996).

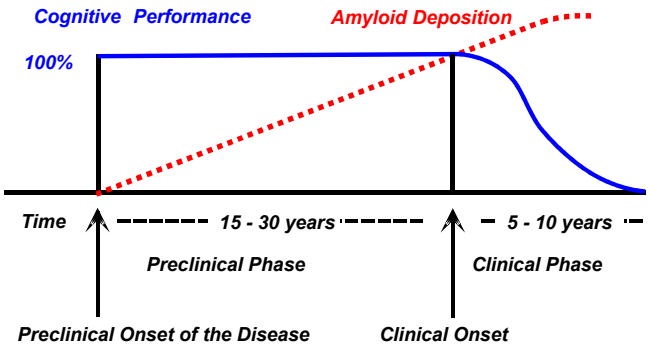

Fig. 3. Neuropathologic events in AD precede clinical onset by 15 to 30 years (Müller-Spahn & Hock, 1999).

Regional cerebral metabolism studies with PET have used 18F-2-deoxy-2-fluoro-D-glucose (FDG) as a metabolic marker to detect these changes. MCI patients show decreased metabolism in the medial temporal lobe region (de Leon et al., 2001; Nestor et al., 2003). In early AD, the most typical pattern is decreased metabolism bilaterally in the parieto-temporal association cortex and cingulate gyrus. This pattern corresponds to the degree of neuropathologic changes in early AD, more prominent in the medial temporal region, cingulate cortex, and in the parietotemporal association cortex (Braak and Braak, 1996; Delacourte et al., 1999). Reiman et al. (1996) were the first to report on the prognostic value of cortical metabolism studied with PET in healthy elderly people with an increased risk of having AD. PSEN1-associated low glucose metabolism has also been found in older nondemented Down's syndrome subjects during brain stimulation (Pietrini et al., 1997). Johnson et al. (2001) used single photon emission computed tomography (SPECT) to measure regional cerebral perfusion. Asymptomatic PSEN1 mutation carriers showed reduced perfusion in comparison to normal noncarrier control subjects in the hippocampal region, anterior and posterior cingulated, posterior parietal lobe and anterior frontal lobe. These studies indicate, that early AD-related changes in brain metabolism and cerebral perfusion are detectable with FDG PET and SPECT, making them the most sensitive neuroimaging methods for detecting preclinical markers of AD. However, by the use of an appropriate cognitive task and fMRI it may become possible, as we shall see later, to reveal the very first

symptoms of AD even before a change of regional glucose metabolism can be detected by means of FDG PET.

Medial temporal atrophy is the most sensitive and specific measure to detect early AD changes on structural brain imaging. In one study entorhinal cortex volume separated normal elderly from those with mild AD with an accuracy of 100% (Killiany et al., 2000). Longitudinal studies have shown, that the annual rate of volume change in entorhinal cortex distinguishes AD from controls with greater sensitivity and specificity than one time measurements (Du et al., 2003). Whereas the annual grey matter loss in normal aging is less than 1%, rates as high as 4% occur in early AD (Thompson et al., 2003). The medial temporal region is also the first one to be affectd in MCI and as the disease progresses, posterior cingulate gyrus and temporo-parietal association cortex are involved (Rusinek et al., 2003; Thompson et al., 2003). Schott et al. (2003) assessed the onset of structural change in FAD. The brains of 5 presymptomatic FAD patients and 20 controls were annually scanned twice or more times. No significant changes were found between patients and controls in baseline measures of whole brain, temporal lobe, or ventricular volume, but averaged volumes of both hippocampi and the entorhinal cortices were 16.6% lower in patients. Moreover, atrophy rates for brain, temporal lobe, hippocampus and entorhinal cortex were significantly increased in patients. A linear extrapolation backward suggested that medial temporal lobe atrophy had started 3.5 years before onset, when all patients were asymptomatic. In another study, Gregory et al. (2005) determined gray matter changes in different cortical regions in genetic cases of AD. Genetic mutations were found to influence the degree and regional pattern of atrophy, and were associated with greater medial temporal lobe atrophy than sporadic cases, suggesting that mutations affecting Aß metabolism selectively increase hippocampal degeneration. Cases with mutations in the PSEN1 gene demonstrated additional increased frontotemporal atrophy. The authors argue, that this effect may be due to the influence of PS1 on tau phosphorylation and metabolism.

Taken together, these studies suggest, that increased medial temporal lobe atrophy is an early feature of AD, and that, pathological atrophy probably is occurring several years before the clinical onset of the disease.

2. Goals and hypotheses of the two experimental studies

The purpose of the two studies reported in this doctoral thesis was to investigate genetic influences on memory performance, memory-related brain activity and neuroanatomy. Furthermore, we tried to detect earliest preclinical markers of AD decades before the clinical onset of the disease.

2.1. Effects of Apolipoprotein E (APOE) variants on memory

In the first study we examined the effects of apolipoprotein E allelic variants in young and healthy subjects. Detrimental effects of APOE4 on cognition and brain physiology have been detected in middle-aged to elderly nondemented and healthy subjects. However, it is unclear whether the reported disadvantages of APOE4 were related to its effects on normal neurophysiologic variability in these subjects, or whether they were strongly connected to a possibly ongoing AD-related neuropathology. Because neurofibrillary tangle formation and amyloid plaque deposition in the MTL start 15-30 years prior to the clinical onset of late-onset sporadic forms of AD, it is conceivable that young human subjects in their twenties are devoid of any such pathology, therefore creating ideal premises for a pathology-independent measurement of APOE4 effects on normal memory neurophysiology. By combining neuroimaging and episodic memory tasks tailored to hippocampal function we addressed the following questions:

- First, does the APOE ε4 allele has an effect on episodic memory performance in young and healthy subjects ?

- Second, do ε4 carriers show altered brain activations in brain areas that mediate episodic memory and that are the first areas to be affected by neuropathological changes in AD ?

- Third, is potentially altered brain activity in ε4 carriers specific for episodic memory or any other cognitive function ?

- Fourth, do ε4 carriers show altered brain morphology, especially in the hippocampus ?

2.2. Effects of the C410Y mutation of the Presenilin 1 (PSEN1) gene on memory

In the second study we sought to detect early preclinical signs of AD in mutation carriers of the FAD-causative gene PSEN1. Since FAD patients present with an early age of onset and a faster and massive progression of AD-related neuropathology, compared to the sporadic AD cases, it is conceivable that early signs of the disease might be detectable already decades before the fully clinical presentation of AD. As yet, preclinical signs of AD have been detected some years prior to the onset of the disease in MCI and prodromal AD patients in studies using neuropsychological assessments and neuroimaging meausures of brain metabolism and structural changes. For a preventive treatment of the disease, however, it is of great importance to detect first disease-related changes as early as possible. Hitherto FDG-PET was the most sensitive method for the early detection of abnormal brain metabolism. There are utterly no studies using functional magnetic resonance imaging to detect preclinical signs of AD in young subjects. We combined neuropsychological testing, functional and structural MRI and sensitive hippocampus-dependent episodic memory tasks to detect earliest AD-related changes in young PSEN1 muation carriers. We hypothesized that:

- a) early changes in memory-related hippocampal activity should be detectable decades before clinical onset of the disease in young AD-causative gene mutation carriers, because PSEN1 mutation carriers might suffer from early deposits in MTL structures elicited by the presence of the mutation since birth

- b) according to the ‘compensatory hypothesis’ young PSEN1 mutation carriers should exhibit enhanced hippocampal and/or neocortical memory-related brain activity when performing sensitive episodic memory tasks to achieve comparable performance levels as noncarriers

- c) young PSEN1 mutation carriers will not show deficits in cognitive functions assuming that the early preclinical regional neuronal dysfunction can be compensated to keep performance at normal levels

3. Original manuscripts

M1 Mondadori, C.R.A., de Quervain, D. J.-F., Buchmann, A., Mustovic, H., Schmidt, C.F., Boesiger, P., Nitsch, R.M., Hock, C., Papassotiropoulos, A., & Henke, K. (2005). Better Memory and Neural Efficiency in Young Apolipoprotein E ε4 Carriers. *Cerebral Cortex, submitted.*

M2 Mondadori C.R.A., Buchmann, A., Mustovic, H., Schmidt, C.F., Boesiger, P., Nitsch, R.M. Hock, C., Streffer, J., & Henke, K. (2005). Memory Impairment and Enhanced Memory-Related Brain Activity in a young Presenilin 1 C410Y Mutation Carrier. *Brain, submitted.*

Better Memory and Neural Efficiency in Young Apolipoprotein E ε4 Carriers

Abrreviated title: Apolipoprotein E ε4 leads to better memory and neural efficiency

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The apolipoprotein E (*APOE*) ε4 allele is the major genetic risk factor for Alzheimer's disease (AD), but an effect on memory in the absence of AD neuropathology is unknown. We found an association of *APOE* ε4 with *better* episodic memory compared to *APOE* ε2 and ε3 in 340 young, healthy persons. Neuroimaging was performed in a subset of 34 memory-matched individuals to study genetic effects on memory-related brain activity independently of differential performance. E4 carriers *decreased* brain activity over three learning runs, while ε2 and ε3 carriers *increased* activity. This smaller neural investment of ε4 carriers into learning reappeared during retrieval: ε4 carriers exhibited reduced retrieval-related activity with equal retrieval performance. *APOE* isoforms had no differential effects on neuropsychological performance, brain volumes, and brain activity related to working memory. We suggest that *APOE* ε4 is associated with good episodic memory and an economic use of memory-related neural resources in healthy humans.

Introduction

The *APOE* ε4 allele (*APOE*4) has an extensive record of deleterious effects on several biological processes. The possession of at least one ε4 allele increases the risk of AD (Saunders et al., 1993) threefold, presumably through an association of *APOE*4 with the pathologic hallmarks of AD, i.e., amyloid plaque deposition and neurofibrillary tangle formation (Bennett et al., 2003; Tiraboschi et al., 2004). In contrast, the possession of an ε2 allele appears to confer a protective effect against AD and AD-related neuropathology (Corder et al., 1994). Moreover, *APOE2* is associated with a reduced decline in episodic memory in elderly persons (Wilson et al., 2002), while *APOE*4 exerts detrimental effects in middle aged and elderly subjects on memory performance (Baxter et al., 2003; Bondi et al., 1995; Hyman et al., 1996), visual attention (Greenwood et al., 2000), executive functions (Rosen et al., 2005), memory-related brain activity (Bondi et al., 2005; Bookheimer et al., 2000) and resting brain glucose metabolism (Reiman et al., 2002b). *APOE*4 has also been experimentally related to impaired hippocampal plasticity (Levi et al., 2003) and lower density of dendritic spines (Ji et al., 2003) in aged transgenic mice and to increased neurotoxicity (Qiu et al., 2003) *in vitro*.

Although the frequency of the *APOE*4 allele in humans is low (15% in Caucasians), studies in primates suggest that it is the ancestral allele (Finch and Sapolsky, 1999). The common (75% in Caucasians) and uniquely human *APOE*3 allele appeared as a mutation and its frequency increased during human evolution (Finch and Sapolsky, 1999). Because the *APOE*4 allele has been related to several deleterious biological effects, the question arises why it existed in the first place (Finch and Sapolsky, 1999). From the evolutionary standpoint, a possible advantageous effect of the *APOE*4 allele in childhood and early adulthood could explain its existence and further persistence in humans. Support for this notion comes from studies where *APOE*4 has been associated with higher IQ scores (Yu et al., 2000), a higher educational level (Hubacek et al., 2001), a reduced cardiovascular response to experimentally induced stress (Ravaja et al., 1997), and a protective effect against spontaneous abortion during embryogenesis (Zetterberg et al., 2002).

It is conceivable that the effects of the *APOE* isoforms in AD may be distinct from their role in normal memory. As yet, an effect of the *APOE* isoforms on normal human memory is unknown. To address this issue, we combined human genetics with memory assessments and functional and structural neuroimaging and mapped genetically transmitted differences in normal memory performance onto brain functions. *Behavioral effects* of the *APOE* gene's three allelic variants on learning and memory were assessed in a large sample of 340 healthy

young subjects (years of age, M: 22.8, SD: 4.0). *Neurophysiologic effects* of the *APOE* gene's allelic variants were examined with functional magnetic resonance imaging (fMRI) in 34 of the 340 original subjects. The 34 subjects underwent one fMRI experiment on episodic memory (Fig. 1) and a second fMRI experiment on working memory. The examination of both episodic and working memory allowed us to determine the specificity of the genetic effects on memory.

Materials and Methods

Subjects. A description of the large subject sample and the genotyping procedures is provided elsewhere (de Quervain et al., 2003). For the *APOE* isoforms, 40 subjects had either the ε2/ε2 or the ε2/ε3 alleles, 214 the ε3/ε3 alleles, 80 the ε3/ε4 alleles and 6 subjects had the ε4/ε4 alleles.

Of the 34 subjects (years of age, M: 22.3, SD: 2.53) who participated in the fMRI experiments, 11 carried the ε2/ε3 genotype, ten the ε3/ε3 genotype, and 13 the ε3/ε4 genotype. Importantly, these subjects were drawn from the large sample such that no statistically significant differences occurred between groups for age, years of education, the His452Tyr polymorphism of the 5-HT2a receptor encoding gene (de Quervain et al., 2003), and the 5-min words recall performance in the previously applied verbal memory test (de Quervain et al., 2003) (Table 1). The reason why we matched the three genotype groups for memory performance was to eliminate performance-related brain activity differences in order to sample only genetic effects on brain activity. The subjects reported neither past nor current psychiatric or neurological problems and denied taking illegal drugs or prescription medication. Their anatomical MRI scans showed normal brain structures. All subjects gave written informed consent to participate in the study after the nature and possible consequences of the study had been explained. The experiments were approved by the ethics committee of the Kanton Zurich.

Table 1. *APOE* subsamples.

APOE	ε2/ε3 (n = 11)	ε3/ε3 (n = 10)	ε3/ε4 (n = 13)
Age	22.7 ± 1.7	21.6 ± 1.7	22.6 ± 3.5
Years of education	14.5 ± 1.3	13.8 ± 1.5	14.3 ± 1.7
Sex (m/f)	2 / 9	4 / 6	7 / 6
Handedness (R/L/A)	11 / 0 / 0	8 / 2 / 0	11 / 1 / 1
His452Tyr	4	4	5
Words delayed recall	7.2 ± 1.7	7.6 ± 2.7	7.7 ± 2.9

Values: means ± SD.

Experimental procedure – large sample. The 340 subjects viewed six sets of words, each set consisting of five semantically unrelated nouns. Subjects were instructed to learn the five words of a set for immediate recall. The immediate recall followed the presentation of each word set. Five minutes following the completion of the six word sets, subjects engaged in a surprise delayed recall test of the 30 learned nouns (de Quervain et al., 2003).

Experimental procedure – small sample. Subjects underwent two fMRI experiments, one on episodic memory and the other on working memory. Trials were blocked in each fMRI experiment. Subjects practiced all fMRI tasks prior to scanning. The same stimuli were used for all subjects to reduce inter-subject variance caused by stimulus-generated effects. Responses were collected with a response box that subjects held in their dominant hand.

fMRI Experiment on episodic memory – Encoding. We presented 16 face-profession pairs for associative learning, 16 faces for single face learning, and 24 head contours without physiognomy in the visual baseline condition (Fig. 1).

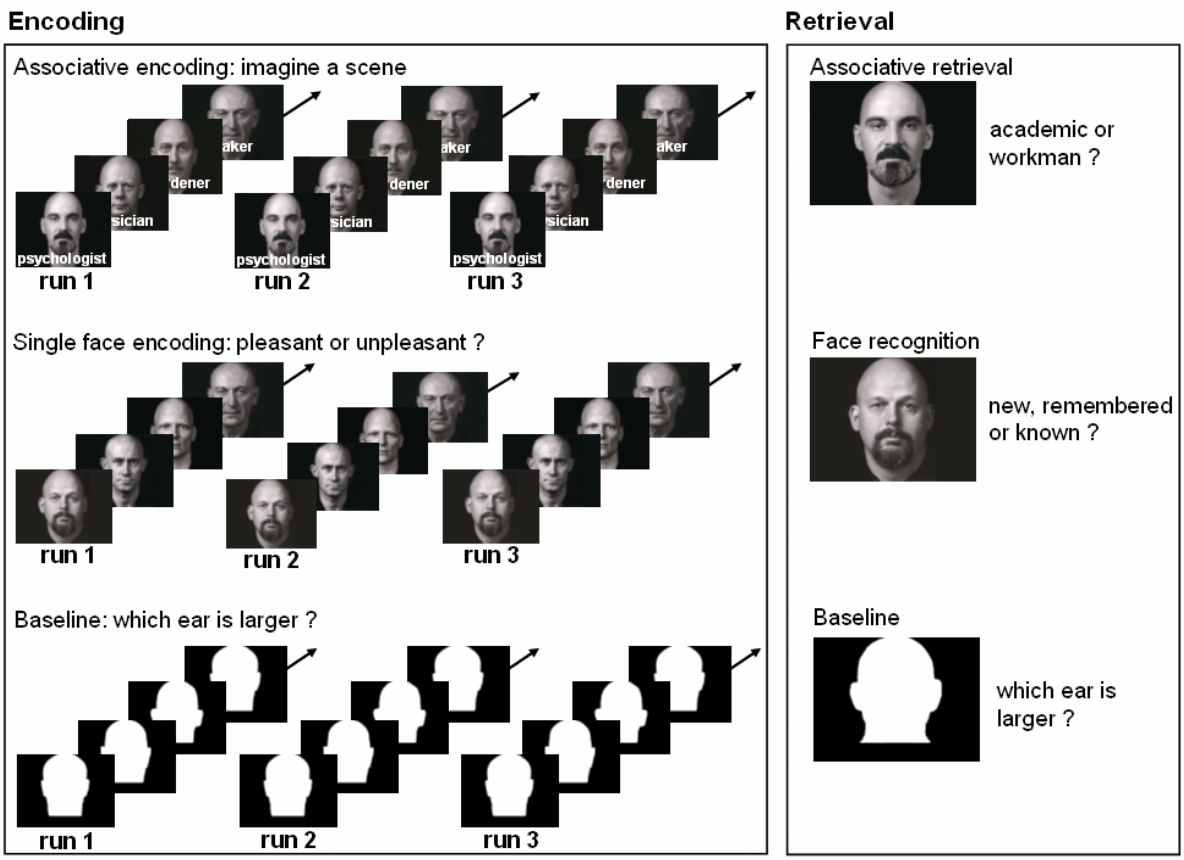

Figure 1. Study design. Left side (encoding fMRI time-series): Examples of stimuli presented for associative learning, for single face learning and for the visual baseline task. Each stimulus appeared once in each run. The order of stimuli was constant across runs. Right side (retrieval fMRI time-series): Examples of stimuli presented for associative retrieval, for single face recognition, for new face detection and for the visual baseline task. Alex Kayser granted us permission to use faces from his book "Heads", 1985, New York: Abbeville Press.

The face profession-pairs and the single faces were learned over three consecutive learning runs (three separate fMRI time series) to monitor genotype-induced brain activity differences during the progressively deeper encoding of the material. The instruction for associative learning of the face-profession pairs was to imagine the presented person acting in a scene of the written profession. Subjects answered by button press whether they found it easy or hard to imagine a scene. The imagination of a scene automatically leads to the establishment of semantic person-occupation associations and activates the hippocampal formation (Degonda et al., 2005). Importantly, subjects were requested to imagine the same scene for a given face-profession pair during runs 2 and 3 as during run 1. This additional retrieval component in runs 2 and 3 was to enhance potential memory-related differences between genotype groups. The instruction for the learning of single faces was to decide whether a face was pleasant or unpleasant. This task yields a semantic encoding of faces. The visual baseline task was to decide whether the area of the left or right ear was larger. The sequence of conditions within the fMRI time-series was counterbalanced across subjects. Each learning condition consisted of four blocks. A block contained four trials of 6 s each. The baseline condition consisted also of four blocks. Here, a block contained six trials of 4 s each. Consequently, each task block took 24 s. An instruction slide announced each task block.

fMRI Experiment on episodic memory – Retrieval. We applied a single fMRI time-series for the retrieval of the previously learned face-profession associations and faces. This time-series included an associative retrieval condition, a face recognition condition, a new faces (distracter faces) detection condition and the same visual baseline condition that was used for the encoding time-series (Fig. 1). For the retrieval of the associations, the previously presented faces were shown again (without the professions) as retrieval cues with the instruction to recall each person's occupation and to indicate the superordinate professional category by button press: academic or workman. For face recognition/new face detection, old (studied) and new faces were presented with the instruction to indicate by button press whether a face was fully recollected, appeared just familiar or was completely new (Tulving, 1985). The sequence of conditions within the fMRI time-series was counterbalanced across subjects. All conditions, except for the visual baseline condition (see above), consisted of four blocks, each block including four trials of 6 s each. All task blocks took 24 s and were announced by an instruction slide.

fMRI Experiment on working memory. The experiment included one fMRI time-series with a 2-back task for the assessment of working memory and a baseline task ('x-target') for the assessment of concentration. The 2-back task required subjects to respond to a

letter repeat with one intervening letter (e.g. **S** – f – **s** – g). The 'x-target' task required subjects to respond to the occurrence of the letter 'x'.

Each task was given in five blocks of 26 s each. Blocks were announced by an instruction slide. Stimuli were 50 upper- or lowercase letters typed in black on a white background. Thirteen upper- or lowercase letters were presented per block for the duration of 2 s each.

Data acquisition. MR measurements were performed on a 3T Philips Intera whole body MR scanner equipped with an eight-channel Philips SENSE head coil. Functional data were obtained from 32 transverse slices parallel to the AC-PC plane covering the whole brain with a measured spatial resolution of 2.8 x 2.8 x 4 mm^3 (acquisition matrix 80 x 80) and a reconstructed resolution of 1.7 x 1.7 x 4 mm^3. Data were acquired using a SENSE-sshEPI (Schmidt et al., 2005) sequence with an acceleration factor of R = 2.0. Other scan parameters were TE = 35 ms, TR = 3000 ms, $\theta = 82°$. A standard 3D T1-weighted scan was obtained for anatomical reference with a measured spatial resolution of 1 x 1 x 1.5 mm^3 (acquisition matrix 224 x 224) and a reconstructed resolution of 0.9 x 0.9 x 0.8 mm^3, TE = 2.3 ms, TR = 20 ms, $\theta = 20°$. A 2D T1-weighted inversion-recovery anatomical scan, oriented perpendicularly to the long axis of the hippocampus, was obtained for hippocampal and parahippocampal volumetry over 33-39 slices with a measured spatial resolution of 0.5 x 0.6 x 1.5 mm^3 (acquisition matrix 400 x 320) and a reconstructed spatial resolution of 0.4 x 0.4 x 1.5 mm^3, TE = 15 ms, TR = 4200 ms, $\theta = 20°$, IR delay 400 ms, and no interslice gaps.

Analysis of functional MRI data. Image pre- and postprocessing and the statistical analyses were performed with SPM2 (http://www.fil.ion.ucl.ac.uk/spm). Standard preprocessing procedures were applied, i.e., realignment, normalization and spatial smoothing (8 mm) (Friston et al., 1995a). On the single subject level, data were analyzed according to the fixed effects model (SPM2). The six head movement parameters were included in the model as confounding factors. Data were high-pass filtered with a specific filter-value for each fMRI time series. This value was determined according to '2*SOA*TR'. On the second level, within-subject contrasts were entered into random effects analyses (ANOVAs, T-tests, SPM2) which account for variance between subjects (Friston et al., 1995b). We also computed correlations between the within-subject encoding contrasts (learning run1 – run3) and behavioral measures (simple regression, SPM2). Thresholds were set at a $p < 0.001$ level, uncorrected for multiple comparisons; exceptions were made for the region of interest, i.e., the hippocampus, and are indicated where applicable.

Analysis of anatomical MRI data. Based on the 3D-T1-weighted structural MRI images which covered the whole brain, volumes of the total gray and white matter were

computed with SPM2. Images were first normalized into the MNI T1 template using a standard bounding box and then segmented into gray matter, white matter and cerebrospinal fluid. Standardized gray and white matter volumes were then multiplied by the determinant of the linear transformation matrix to obtain gray and white matter volumes in cm^3. Based on the 2D-T1-weighted high resolution structural MRI images, two independent raters (A.B. and H.M.) manually delineated the hippocampal formation (Henke et al., 1999a) (CA regions, dentate gyrus and subiculum, excluding the fimbria) and the parahippocampal gyrus using the software Pmod (http://www.pmod.com.). Cerebrospinal fluid was carefully excluded resulting in conservative volume estimates. Raters relied on descriptions of anatomical landmarks and subdivisions of the MTL as described by Insausti et al. (1998) and Duvernoy (1998). Inter-rater reliabilities ranged between r = 0.8 and 0.98. ANOVAs with *APOE* genotype and sex as independent variables were computed to determine group differences in brain volumes. Thresholds were set at $p < 0.05$ level, uncorrected for multiple comparisons (6 subjects excluded from this analysis because of data loss).

Neuropsychology. Memory functions were assessed with the Wechsler Memory Scale Revised (Härting et al., 2000) in German and with and a difficult verbal paired-associates word learning test (VAT) (Henke et al., 1999b). Intelligence was measured with the Hamburg Wechsler Intelligence Test (Tewes, 1991). Spatial thinking abilities were tested with the Luria Mental Rotation Test (Christensen, 1979). Executive functions were assessed with a verbal (S-Words) and nonverbal (5-Point) fluency task (Regard et al., 1982), with the Kramer Card Sorting Test (Kramer, 1970), which measures concept finding and shifting abilities, and with the Stroop Test (Stroop, 1935), which measures the suppression of interference. ANOVAs with the *APOE* genotype as an independent variable were computed to determine group differences in cognitive performance. Thresholds were set at $p < 0.05$ level, uncorrected for multiple comparisons (one subject excluded from this analysis because of non-compliance).

Results

Behavioral results – large sample

The *APOE*4 carriers, compared to non-carriers, exhibited better performance in the delayed, but not the immediate recall of the 30 studied words (odds ratio = 1.6, P = 0.009; Fig. 2A). This result remained significant after excluding the six homozygous ε4 allele carriers from the analysis (odds ratio = 1.6, P = 0.023). Moreover, multiple linear regression

analysis revealed a significant linear relationship between the presence of no, one, or two ε4 alleles and the delayed recall of words ($\beta_{standardized}$= 0.118, P = 0.023; Fig. 2B).

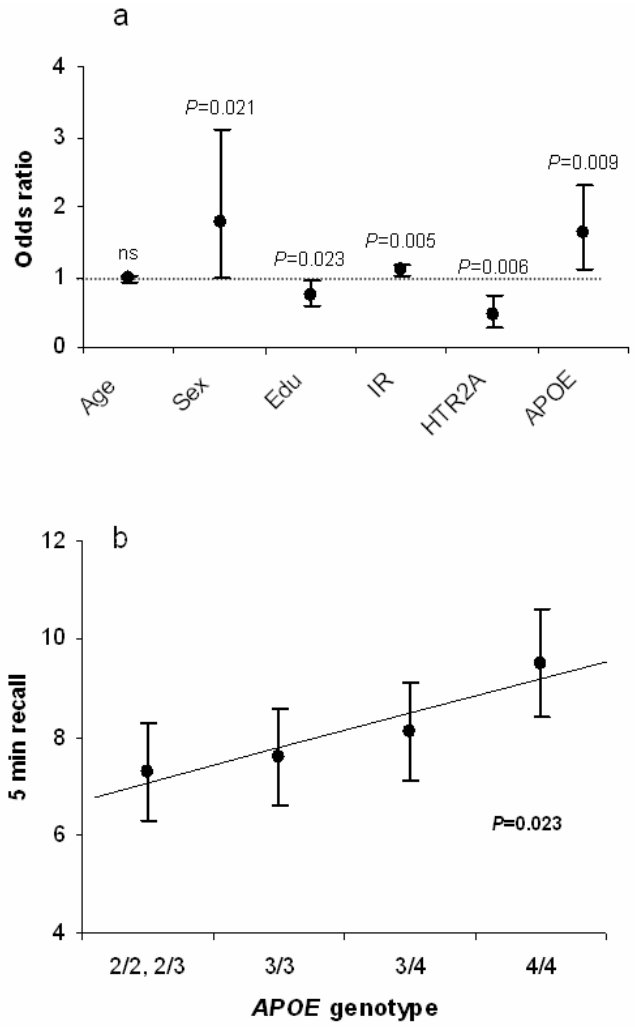

Figure 2. APOE effects on verbal memory in 340 subjects. a) Forward and backward logistic regression analysis after median split for the 5-min recall of words (dependent variable). Factors significantly associated with better memory performance (> median of 8) were female sex, good immediate recall (IR) and the APOE ε4 allele, while age, education (Edu) and the H452Y substitution of the HTR2a gene were not. Dotted line indicates odds ratio of 1. Error bars reflect 95% confidence interval of odds ratio per independent variable. b) Multiple forward and backward linear regression with the 5-min recall performance as dependent variable adjusted for sex, education, immediate recall and the H452Y substitution of the HTR2A gene. Dots depict mean, bars depict standard error of mean (SEM).

These performance differences in the delayed recall test cannot be attributed to differences in working memory, motivation, or attention because the immediate recall of words was comparable between the genotype groups. To minimize the possibility of a type I error due to non-random genetic heterogeneity of the study sample, we computed the genetic structure of the population by genotyping each subject for 55 unlinked single nucleotide polymorphisms

located mostly in non-genic regions and distributed over all autosomes. Structured association analysis (Pritchard and Rosenberg, 1999) revealed a low allele-frequency divergence in our population (Kullback-Leibler distance = 0.15) and excluded non-random, gross genetic heterogeneity as a potential source of false positivity.

Behavioral results – small sample

Response latencies decreased significantly from learning run1 to run3 within each *APOE* group for both faces and face-profession pairs supporting effective learning in each *APOE* group. *APOE* group differences in response latencies occurred neither within nor between runs (Table 2). The number of 'easy' (to imagine) answers during associative learning increased over learning runs for each *APOE* group (Table 2; effect did not quite reach significance in *APOE* ε3/ε4 carriers with $p = 0.06$). This facilitation of imagining a scene was also indicative of effective learning over runs. There were no *APOE* group differences in the number of 'easy' answers within or between runs. The number of 'pleasant' decisions for the single faces remained constant over the three learning runs for each *APOE* group. *APOE* groups did not differ in the number of given 'pleasant' answers within or between runs (Table 2).

Retrieval accuracies and response latencies for faces and face-profession associations were statistically equal between *APOE* groups (Table 2).

In the working memory experiment, neither accuracy nor reaction latency differed significantly between *APOE* groups for the 'x-target' and the 2-back task (Supplemental Table S1).

Performance measures acquired during the neuropsychological examination of the 34 subjects - i.e., measures of memory, intelligence, spatial cognition and executive functions - were also statistically equal across *APOE* groups (Supplemental Table S2).

Table 2. ANOVA on the groups' latency and accuracy values in the learning and retrieval tasks.

	ε2/ε3	ε3/ε3	ε3/ε4	F	P
Associative learning					
# easy (run1)	8.9 ± 2.2	8.6 ± 2.5	8.8 ± 2.1	0.5	0.951
# easy (run2)	9.3 ± 2.8	9.9 ± 2.6	8.9 ± 1.8	0.465	0.632
# easy (run3)	10.9 ± 1.9	10.4 ± 2.9	10.8 ± 2.9	0.104	0.902
# easy (run1-run3)	-2 ± 2*	-1.8 ± 2.3*	-1.7 ± 2.8	0.056	0.946
RT run1	3212.6 ± 841	3179.1 ± 599.5	3164.5 ± 624.9	0.015	0.985
RT run2	2944.8 ± 922	2934.4 ± 514.4	2884.2 ± 639.9	0.261	0.772
RT run3	2628.5 ± 798.5	2725 ± 706.4	2717.8 ± 840.3	0.117	0.89
RT run1-run3	584 ± 633.3*	454.1 ± 607*	472.9 ± 507.5*	0.158	0.855
RT run1-run2	267.7 ± 595.7	244.7 ± 423	366.1 ± 332.8*	0.221	0.803
RT run2-run3	316.3 ± 253.1*	209.5 ± 358.1*	116.1 ± 331.2	1.107	0.344
Single face learning					
# pleasant (run1)	6.8 ± 2.9	7.2 ± 1.2	7.9 ± 3	0.566	0.573
# pleasant (run2)	7.2 ± 3.2	6.8 ± 2.3	7.7 ± 2.8	0.267	0.768
# pleasant (run3)	7.5 ± 2.9	7.1 ± 2	7.5 ± 3.3	0.062	0.94
# pleasant (run1-run3)	-0.6 ± 1.3	0.1 ± 1.2	0.5 ± 1.4	1.758	0.19
RT run1	2132.9 ± 511.9	1945.2 ± 227	2081.5 ± 324.4	0.699	0.505
RT run2	1924.5 ± 477.9	1944.3 ± 343.4	1963.2 ± 402.1	0.025	0.975
RT run3	1541.8 ± 508.9	1702.5 ± 312.4	1581.6 ± 388.6	0.426	0.657
RT run1-run3	591.1 ± 392.8*	242.7 ± 243.4*	490.3 ± 500.4*	2.075	0.143
RT run1-run2	208.4 ± 340.1*	0.9 ± 222.1	153.4 ± 345.3	1.237	0.305
RT run2-run3	382.7 ± 207.2*	241.7 ± 244.4*	314.4 ± 273.9*	0.879	0.426
Associative retrieval					
# correct	12.3 ± 2.2	11.4 ± 2.3	12.2 ± 2.4	0.442	0.647
RT correct answers	2597.1 ± 338.6	2621.9 ± 425.8	2888.1 ± 413	1.928	0.163
Single face retrieval					
# correct remember *(hits - false alarms)*	10.2 ± 4.5	10.3 ± 3.1	10.2 ± 4.6	0.003	0.997
RT hits remember	1609 ± 428	2142.2 ± 415	2163.7 ± 856.1	2.857	0.073
# know *(hits - false alarms)*	2.5 ± 4.0	3.4 ± 3.2	3.3 ± 5.9	0.121	0.887
RT hits know	2513.4 ± 823	2666.1 ± 594.9	2709.7 ± 538.3	0.248	0.782

Values: means ± SD, reaction times (RT) in milliseconds, * one sample t-test: difference is significant within group, $p < 0.05$

Imaging results – small sample

The analysis of the imaging data focused on the medial temporal lobe (MTL). One-sample T-tests including all 34 subjects revealed robust bilateral hippocampal activation for face-profession associative learning and for single face learning confirming that our tasks activated the hippocampus reliably in the present subjects (data not shown). One-way ANOVAs (SPM2) with the *APOE* genotype as an independent variable were followed by two sample T-tests between the ε3/ε4 and ε2/ε3 carriers. We directly compared the ε3/ε4 and ε2/ε3 carriers

because the ε4 and the ε2 allele might not only confer opposite effects on the risk of AD (Corder et al., 1994) but also on the normal memory-related physiology. Therefore, we were expecting to see the largest brain activity differences between the ε3/ε4 and the ε2/ε3 carriers.

Significant differences in signal change from learning run1 to run3 in *both* the ANOVA and the post-hoc t-tests between the ε3/ε4 and the ε2/ε3 carriers were located in bilateral hippocampus during associative learning and in the left hippocampus during single face learning (Table 3, Fig. 3A).

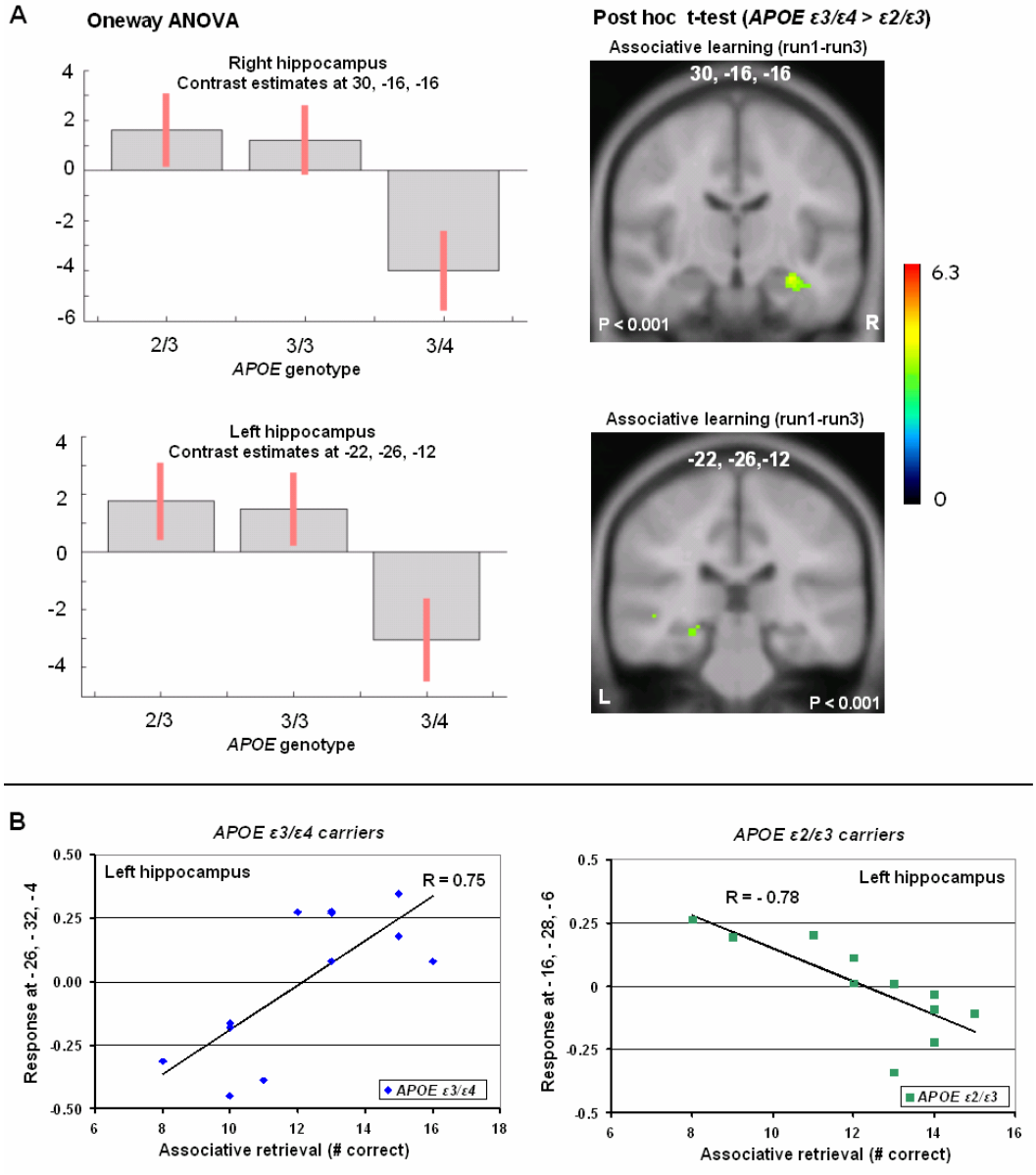

Figure 3. *APOE* effects on learning-related brain activity in 34 subjects. a) Left panel: One-way ANOVA showing *APOE* effect on the right and left hippocampal signal change from run1 to run3 of associative learning (run3 - run1). Columns indicate mean-corrected parameter estimates, bars depict SEM parameter estimates. Right panel: Larger right and left hippocampal signal decrease during associative learning from run1 to run3 in

APOE ε3/ε4 versus *APOE* ε2/ε3 carriers displayed as color-coded statistical parametric t-map overlaid on Montreal Neurologic Institute (MNI) anatomical scan. b) Simple regression between left hippocampal signal difference between runs (run1 – run3) during associative learning and the retrieval success computed for *APOE* ε3/ε4 (left panel) and *APOE* ε2/ε3 carriers (right panel).

Further effects were situated in neocortical areas, namely the left orbital gyrus and a left posterior middle temporal region during associative learning and in the middle frontal gyrus during single face learning (Table 3).

While the ε3/ε4 carriers *decreased* their learning-related hippocampal and neocortical activity from the first to the third learning run, the ε2/ε3 and the ε3/ε3 carriers *increased* their learning-related hippocampal and neocortical activity from the first to the third learning run (Fig. 4).

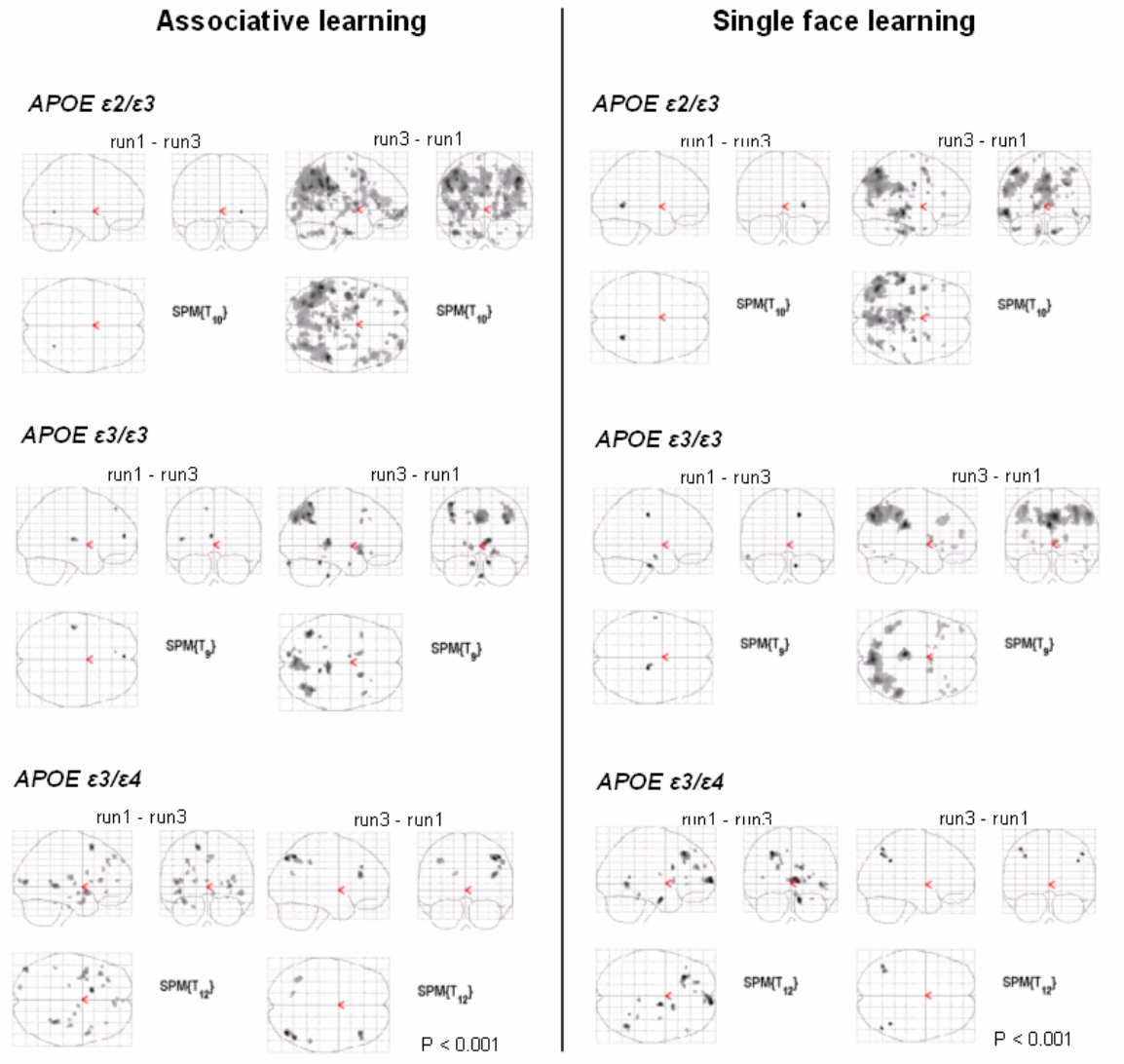

Figure 4. Whole brain activity differences between learning runs 1 and 3 for each *APOE* group. Sagittal, coronal and horizontal SPM2 through-projections of brain regions that exhibited a significant signal decrease (run1-run3) or signal increase (run3 – run1) during associative learning (left side) and single face learning (right side).

These differences in the slope of activity changes over learning runs were not caused by unequal starting levels of learning-related activity between groups, because there were no significant brain activity differences between *APOE* groups in the first learning run (data not shown).

Next, we computed correlations between the signal change from learning run1 to run3 and the number of correctly remembered associations or faces in order to determine whether the slope of learning-related brain activity was related to retrieval success. These correlations were *positive* (i.e., the more signal decrease during learning, the better the retrieval performance) for the ε3/ε4 carriers, but *negative* (i.e., the more signal increase during learning, the better retrieval performance) for the ε2/ε3 carriers within the left hippocampus (ε3/ε4: r = 0.75; ε2/ε3: r = - 0.78; Fig. 3B) and several left and right prefrontal areas.

During retrieval, the ε3/ε4 carriers exhibited sparser retrieval-related neural recruitment than the ε2/ε3 carriers in spite of their equivalent retrieval success. The comparison of the retrieval of face-profession associations with the retrieval of single faces isolates brain activity underlying the retrieval of associations alone. In this comparison, the ε3/ε4 carriers exhibited weaker activity compared to the ε2/ε3 carriers in the right hippocampus and the left fusiform gyrus (Table 3). In the comparison of single face retrieval with baseline, the ε3/ε4 carriers exhibited weaker activity increases compared to the ε2/ε3 carriers in the right middle and superior frontal gyri and in the right precuneus (Table 3).

The reported brain activity differences between *APOE* groups were specific to episodic memory because the working memory fMRI experiment yielded no MR signal differences between the ε2/ε3 and the ε3/ε4 carriers.

Table 3. Brain activation differences between *APOE* genotype groups during repeated learning and retrieval. Listed are regions significant in both the one-way ANOVA including the three genotype groups and the post hoc t-tests between ε3/ε4 and ε2/ε3 carriers.

		MNI Coordinates (mm)					
Brain Region	**Left/Right**	**x**	**y**	**z**	**^{k}E**	**t**	**F**
Oneway ANOVA & post hoc t-tests							
Repeated associative learning (run1 > run3)							
APOE ε3/ε4 > ε2/ε3							
Hippocampus	R	30	-16	-16	8		7.66
	R	30	-18	-16	182	4.29	
	L	-22	-26	-12	9		7.07[a]
	L	-22	-26	-12	15	4.01	
Orbital gyrus	L	-24	28	-8	21		10.03
	L	-24	30	-8	11	3.95	
Post. middle temporal area	L	-40	-54	4	71		14.67
	L	-40	-54	4	232	6.34	
APOE ε2/ε3 > ε3/ε4	no significant differences						
Repeated single face learning (run1 > run3)							
APOE ε3/ε4 > ε2/ε3							
Hippocampus	L	-22	-28	-6	6		6.03[b]
	L	-22	-28	-6	3	3.7	
Middle frontal gyrus	L	-30	34	38	25		10.39
	L	-30	34	38	73	5.14	
APOE ε2/ε3 > ε3/ε4	no significant differences						
Associative retrieval > face recognition							
APOE ε3/ε4 > ε2/ε3	no significant differences						
APOE ε2/ε3 > ε3/ε4							
Hippocampus	R	36	-40	-4	13		8.85
	R	34	-40	-6	16	4.01	
Fusiform gyrus	L	-42	-36	-22	17		10.20
	L	-44	-34	-24	4	3.68	
	L	-28	-72	-8	6		6.49[b]
	L	-28	-72	-8	10	4.01	
Single face retrieval > baseline							
APOE ε3/ε4 > ε2/ε3	no significant differences						
APOE ε2/ε3 > ε3/ε4							
Middle frontal gyrus	R	36	26	52	102		11.55
	R	36	26	52	71	4.55	
Superior frontal gyrus	R	6	40	54	1408		20.78
	R	6	40	54	10	3.7	
Precuneus	R	8	-48	50	10		7.66
	R	8	-48	50	122	4.21	

t, F: values of peaks within significantly activated clusters of voxels; post., posterior
[a] $p < 0.002$, [b] $p < 0.003$; [k]E: cluster size (in voxels)

To determine whether *APOE* group differences in brain activity resulted from interindividual differences in the volume of gray or white brain matter, we performed volumetric analyses of the subjects' brain structures. Both automated whole brain gray and white matter segmentation (SPM2) (Ashburner and Friston, 2000) and manual volume measurements of the hippocampal formation and the parahippocampal gyrus yielded comparable volumes between the three *APOE* groups (Table 4).

Table 4. Brain volumes (in cm^3).

	ε2/ε3 (n= 8)	ε3/ε3 (n=7)	ε3/ε4 (n=13)	F	P
Left hippocampus	2.42 ± 0.2	2.7 ± 0.19	2.58 ± 0.34	1.081	0.356
Right hippocampus	2.44 ± 0.22	2.67 ± 0.27	2.59 ± 0.31	0.825	0.451
Left parahippocampal gyrus	4.89 ± 0.76	5.46 ± 0.61	5.19 ± 0.53	1.567	0.23
Right parahippocampal gyrus	4.78 ± 0.71	5.27 ± 0.62	5.29 ± 0.58	0.544	0.587
Whole brain (grey & white matter)	1224.8 ± 90.19	1117.12 ± 162.93	1177.06 ± 153.89	0.187	0.831
grey matter	635.68 ± 57.98	696.05 ± 98.13	720.53 ± 101.81	0.279	0.759
white matter	389.18 ± 39.92	421.08 ± 66.45	456.53 ± 57.84	0.124	0.884
corticospinal fluid (CSF)	215.37 ± 29.71	240.71 ± 34.11	252.96 ± 45.78	0.231	0.795

Displayed are values of both raters (means ± SD), uncorrected

Discussion

*APOE*4 is the major genetic risk factor for AD. Studies in primates suggest that *APOE*4 is the ancestral allele (Finch and Sapolsky, 1999). Its existence and further persistence – though at a low frequency - in humans might be explained by an advantageous effect of *APOE*4 in early adulthood. Here, we report an association of *APOE*4 with better memory performance and with less learning- and retrieval-related brain activity compared to *APOE2* and *APOE*3 in young and healthy human subjects.

In particular, we found a reverse relationship of learning/retrieval success with brain activity levels in *APOE*4 compared to *APOE2* carriers. Although the starting levels of learning-related brain activity were comparable between *APOE* groups, ε3/ε4 carriers *decreased* their learning-related hippocampal and neocortical activity during the progressively deeper encoding of the material, while the ε2/ε3 and the ε3/ε3 carriers *increased* their learning-related brain activity (Fig. 4). This disparity in the learning-related brain activity slopes was not related to group differences in the cognitive processes applied during learning because all performance measures collected during the learning runs were statistically equal between *APOE* groups (Table 2). We therefore suggest that the *APOE* ε4 allele is associated with a more economic use of learning-related hippocampal and neocortical resources.

The question arises of whether the group differences in brain activity slopes reflect a differential neurophysiology underlying episodic memory or another form of memory which is simultaneously active during the learning runs. The repeated exposure to material does not only evoke retrieval (from previous exposure) and re-encoding processes in the realm of episodic memory but also priming. Priming is a form of implicit/nondeclarative memory that involves a facilitation of the ability to identify, produce or classify an item as a result of a previous encounter with that item or a related item (Schacter et al., 2004). Priming is often associated with accelerated information processing (shortening of reaction latencies) and decreasing neural activity over repeated exposures, as observed in our *APOE*4 group. It could therefore be speculated that the differential hippocampal activity slopes over learning runs reflected priming more than episodic memory. We found that neither of the *APOE* groups exhibited a significant correlation of medial temporal activity slopes with the shortening of reaction latencies from run1 to run3 (data not shown). Instead, medial temporal activity slopes were correlated with retrieval success for both faces and face-profession associations. Importantly, these correlations were *positive* - i.e., the more signal decrease over runs, the better retrieval performance - for the ε3/ε4 carriers, but *negative* – i.e., the more signal increase over runs, the better retrieval performance - for the ε2/ε3 carriers (Fig. 3B). These findings indicate that medial temporal activity changes over learning runs were related to episodic memory. At the same time, these correlations also underscore the reverse relationship between episodic memory and hippocampal activity levels in *APOE*4 versus *APOE2* carriers: While a large signal *increase* denoted the good learner among the *APOE2* carriers, a large signal *decrease* during repeated learning characterized the good learner among the *APOE*4 carriers. Together, *APOE*4 carriers engaged less neural resources into the complete learning process compared to *APOE2* carriers which points to a more economic use of neural resources. It is interesting that steeper medial temporal activity slopes over repeated face learning have also been found for healthy subjects compared to patients with an amnestic mild cognitive impairment (Johnson et al., 2004). Thus, it appears that economic use of learning-related medial temporal resources characterizes a well functioning episodic memory system.

We again observed a more economic use of memory-related neural resources in *APOE*4 carriers at the time of retrieval. *APOE*4 carriers invested fewer hippocampal and neocortical activity into the retrieval process than *APOE2* carriers, while achieving an equivalent retrieval success (Table 3). This finding suggests that task demands were higher on the *APOE2* than the *APOE*4 carriers – a finding that is intuitive given the better retrieval

performance in the *APOE*4 carriers in the large and non-matched sample of 340 subjects. Although we had matched participants of the fMRI study for memory performance, task demands might still have been higher on the *APOE2* than the *APOE*4 carriers. It has been demonstrated for a variety of cognitive tasks that increasing task demands are associated with an increase in the spatial extent and the magnitude of brain activity in the task-specialized neural network (Carpenter et al., 1999; Just et al., 1996). Patients with an amnestic mild cognitive impairment exhibited larger memory-related activity enhancements within the medial temporal lobe (Dickerson et al., 2004) compared to healthy individuals. In addition, neuroimaging studies have shown that the brains of high performers or people with much practice work more efficiently (less activity with better task performance) than the brains of lower performers or people with less practice (Haier et al., 1992a; Haier et al., 1992b; Neubauer et al., 2005). We conclude, therefore, that medial temporal activity may be elevated in response to increasing task demands caused by either an objective increase in task difficulty or a subjective increase in task difficulty due to less practice or less favorable genetic or neurophysiologic setting.

It should be noted that our neuroimaging *APOE* groups were statistically equal in terms of neuropsychological test performance (Table S2) and gray and white matter volumes (Table 4) - particularly hippocampal and parahippocampal volumes. It is, therefore, unlikely that the performance and imaging results were biased by morphological or cognitive differences between *APOE* groups. Moreover, brain activity differences between *APOE* groups were found only for episodic memory, not working memory. The three *APOE* groups differed neither in performance (Table S1) nor brain activity levels on the working memory task.

The more economic use of neural resources in *APOE*4 carriers concurs with advantageous effects of the *APOE*4 allele on normal neurophysiologic functioning in young animals. Hippocampal long-term potentiation (LTP) is enhanced at a younger age in knock-in mice lacking mouse *APOE*, but instead expressing human *APOE*4 (Kitamura et al., 2004). This LTP enhancement is age-dependent and disappears in adult knock-in mice. Moreover, *APOE*4, but not *APOE*3, stimulates the transcriptional activity of cAMP-response element-binding protein (CREB) by activating the extracellular signal regulated kinase (ERK) cascade in rat primary hippocampal neurons (Ohkubo et al., 2001). The influence of *APOE*4 on this memory-related pathway may provide an explanation for the beneficial effect of *APOE*4 on human memory in young adults. On the other hand, there is evidence that *APOE*4 alters intracellular calcium homeostasis which might ultimately lead to neuronal damage (Qiu et al.,

2003). One may speculate that a sustained *APOE*4-related neuronal calcium increase, which is related to improved memory performance at a young age, finally induces age-associated neuronal damage.

Alternatively, the *APOE*4-associated cognitive impairment and abnormal brain activity/metabolism found in middle-aged and elderly subjects might be a consequence of the continuous deposition of amyloid plaques and neurofibrillary tangles, which are associated with dysfunction, deafferentation, and degeneration of neurons in the long course of AD. The deposition of amyloid plaques and neurofibrillary tangles has been related to the presence of the *APOE*4 allele (Bennett et al., 2003; Tiraboschi et al., 2004). Importantly, these neuropathological changes precede the clinical diagnosis of AD by 30-50 years (Braak and Braak, 1996; Delacourte et al., 1999; Ohm et al., 1995). A single copy of the *APOE*4 allele in middle-aged and elderly persons with normal memory performance was associated with lowered parietal and temporal metabolism, which predicted cognitive and metabolic decline after two years of longitudinal follow-up (de Leon et al., 2001; Small et al., 2000). Moreover, elderly *APOE*4 carriers, compared to *APOE*3 carriers, exhibited increases in brain activity in response to verbal (Bookheimer et al., 2000) and picture (Bondi et al., 2005) learning presumably to compensate for AD-related preclinical neuropathology. Indeed, the greater their brain activity increase was, the greater was their memory decline during the following two years (Bookheimer et al., 2000). Thus, it appears that the brain activity abnormalities in middle-aged and elderly *APOE*4 carriers reflect on-going pathophysiologic processes of AD. Because of the long preclinical course of AD (Braak and Braak, 1996; Delacourte et al., 1999; Ohm et al., 1995), a direct effect of the *APOE* isoforms on normal memory-related neurophysiology in the absence of disease can only be studied in young subjects.

In conclusion, we suggest that the *APOE*4 allele is associated with positive effects on episodic memory in healthy individuals. Its association with cognitive impairment and abnormal brain activity later in life is probably mediated by AD-related neuropathology. Future studies need to address a potential link between the *APOE*4 allele's beneficial effects on the normal memory neurophysiology and its role as a risk factor for AD.

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Supplemental Data

Table S1. Groups' accuracy and latency values in the working memory task.

	ε2/ε3	ε3/ε3	ε3/ε4	F	P
2-back (hits - false alarms)	9.8 ± 1.8	7.7 ± 3.6	8.5 ± 2.4	1.765	0.189
RT 2-back (hits)	676.5 ± 149.9	724.5 ± 152.4	655.7 ± 98.4	0.729	0.491
RT 2-back (false alarms)	796.7 ± 207.2	857.6 ± 180.5	793.5 ± 294.4	0.202	0.818
x-target (hits - false alarms)	12.9 ± 0.3	13 ± 0	12.8 ± 0.8	0.486	0.62
RT x-target (hits)	507.5 ± 68.7	558.2 ± 82.4	484.7 ± 76.3	2.593	0.91
RT x-target (false alarms)	401 ± 0		574 ± 0		

Values: means ± SD, reaction times (RT) in milliseconds

Table S2. Results neuropsychological examination.

	ε2/ε3 (n=11)	ε3/ε3 (n=10)	ε3/ε4 (n=12)	F	P
IQ (WAIS)	123.3 ± 12	121.6 ± 8.6	126.4 ± 8.9	0.704	0.503
WMS-R					
verbal memory	117.1 ± 12.7	109.1 ± 12.7	111.1 ± 14.2	0.985	0.385
visual memory	118 ± 6.9	117.8 ± 7	117 ± 9	0.45	0.956
general memory	120.2 ± 11.7	113.6 ± 11.3	114.9 ± 12.2	0.904	0.416
concentration & attention	100.3 ± 10.9	105.2 ± 14.6	108.5 ± 15	1.014	0.375
delayed recall	119.6 ± 9.5	116.3 ± 10.1	112.9 ± 15.7	0.811	0.454
VAT					
recall (# correct)	7.6 ± 3	6 ± 5.3	8.5 ± 4.6	0.935	0.404
recognition (hits - false alarms)	7.9 ± 1.9	6.6 ± 2.1	8.5 ± 2.1	2.411	0.107
Luria mental rotation	9.4 ± 0.7	9.6 ± 0.7	9.6 ± 0.7	0.33	0.722
Kramer card sorting	4.5 ± 1	5.2 ± 1.1	5.4 ± 0.5	3.043	0.063
Stroop* color interference	21.1 ± 5.3	21.5 ± 5.7	20.8 ± 5.8	0.046	0.955
S-Words fluency	36 ± 6.1	32.9 ± 8.4	33.9 ± 8.5	0.419	0.662
5-Point nonverbal fluency	41.8 ± 5.9	38.5 ± 8.4	45.9 ± 7.7	2.866	0.073

Values: means ± SD, * seconds, VAT verbal associative learning test, WAIS Wechsler Adult Intelligence Scale, WMS-R Wechsler Memory Scale Revised

Memory Impairment and Enhanced Memory-Related Brain Activity 30 Years Before the Manifestation of Alzheimer's Disease

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Summary

Presenilin 1 (*PSEN1*) mutations cause familial Alzheimer's disease (FAD) in an autosomal dominant mode of transmission. *PSEN1* mutation carriers exhibit the normal course of cognitive deterioration seen in sporadic Alzheimer's disease (AD) with first deficits in episodic memory. However, FAD is associated with a younger age of onset and a faster disease progression compared to sporadic AD. Here, we sought to determine preclinical signs of AD decades before the clinical onset of the disease. Five *nondemented* members of a family with a *PSEN1* mutation (C410Y) as well as 21 controls from non-AD families were examined, using functional and structural magnetic resonance imaging and neuropsychological assessments. A 20 year old and a 45 year old family member carried the C410Y mutation on the *PSEN1* gene. The age of AD diagnosis in this family is around 48 years. Both mutation carriers exhibited a selective impairment (> 2 SDs) in tasks of episodic memory. We found an enhanced neural recruitment, compared to controls and family members without the mutation, in a memory-related network during both episodic learning and retrieval in the 20 year old mutation carrier. The 45 year old mutation carrier exhibited decreased brain activity in memory-related areas during episodic learning and retrieval. The amplified activity in the 20 year old subject might reflect a compensatory effort to overcome preclinical memory dysfunction induced by accumulating AD pathology, while activity reductions in the 45 year old subject might reflect gross neural dysfunction in a more advanced stage of neuropathology. We suggest that Alzheimer-related memory dysfunction can be identified 30 years before AD diagnosis in individual subjects with the use of functional neuroimaging and memory tasks tailored to MTL function.

Keywords: Presenilin 1, Alzheimer's disease, fMRI, memory, hippocampus

Introduction

Familial Alzheimer's Disease (FAD) is transmitted in an autosomal dominant manner and has been associated with mutations in the amyloid precursor protein gene (*APP*) on chromosome 21 (Goate et al., 1991), the presenilin 1 gene (*PSEN1*) on chromosome 14 (Alzheimer's Disease Collaborative Group, 1995; Sherrington et al., 1995) and the presenilin 2 gene (*PSEN2*) on chromosome 1 (Levy-Lahad et al., 1995; Rogaev et al., 1995). Approximately 55% of all FAD cases are caused by mutations in the *PSEN1* gene (Cruts and van Broeckhoven C., 1998). Today, more than 50 missense mutations of the *PSEN1* gene are known with an age of onset of AD ranging from 25 years to 64 years (Czech et al., 2000). The first clinical signs in early AD are episodic memory deficits and declines in executive and visuospatial functions. In comparison to sporadic forms of AD (SAD), familial cases show initial clinical symptoms at an earlier age and have a faster disease progression along with more abundant tissue histopathology (Gomez-Isla et al., 1997; Lippa et al., 2000; Sakamoto et al., 2002). The neuropathological course of AD is characterised by a broad cortical amyloid beta (Aß) deposition and tau pathology (neurofibrillary tangles) starting prominently in the medial temporal lobe (MTL) region 15-30 years prior to clinical symptoms, then spreading throughout the neocortex in later stages of the disease (Braak and Braak, 1996; Delacourte et al., 1999; Price et al., 1991). It is of great importance that preventive therapies can be developed which might slow down or even stop the process of neuropathological deterioration. For an optimal treatment of the disease the availability of valid neuropsychological, neuroanatomical, neurophysiological or biological markers for the early diagnosis is mandatory.

As yet, one of the most sensitive methods in the early diagnosis of AD is fluorodeoxyglucose positron emission tomography (FDG PET). Metabolic changes have been detected in cognitively normal carriers of the apolipoprotein E ε4 allele (*APOE4*), a risk allele for AD (de Leon et al., 2001; Reiman et al., 2004; Reiman et al., 2005; Reiman et al., 1996; Small et al., 2000). Abnormally low rates of glucose metabolism were found in the same brain regions as in patients with probable AD, i.e. in posterior cingulate, parietal, temporal and prefrontal cortex, even in young adulthood (Reiman et al. 2004). Notably, in some cognitively healthy subjects at risk for AD, cerebral metabolism at rest was normal, but a stress test paradigm (audio-visual stimulation) revealed temporo-parietal metabolic abnormalities (Pietrini et al., 1997), i.e. affected neurons showed some malfunction when activated by an appropriate task.

Task-induced activity alterations in persons at risk for AD were also found in studies

with functional magnetic resonance imaging (fMRI). Bookheimer et al. (2000) and Bondi et al. (2005) found greater magnitude and extent of brain activation in healthy elderly carriers of *APOE4* during memory-activation tasks in regions affected by AD, including the hippocampal, temporal, parietal, and prefrontal regions. The authors argue that such increased brain activity may effectively serve a compensatory role, wherein subjects use additional cognitive resources to bring memory-related performance to a normal level. However, *APOE4* carriers exhibited already lower performance scores on one of the four delayed recall tests than did carriers of the *APOE3* allele at the initial neuropsychological testing (both groups still fell into the normal range for this age group) and developed memory decline after two years. We believe that functional changes in memory-associated brain structures should occur far earlier than only two years before clinical onset. This hypothesis is supported by two longitudinal studies which document the cognitive profiles of subjects who later developed AD over a period of 6 years before clinical onset (Backman et al., 2001; Fox et al., 1998). Both studies showed significantly lower scores of verbal episodic memory in individuals who manifested clinically within 6 years compared to subjects who remained healthy. These findings imply that cognitive decline can precede the clinical phase as long as 6 years. However, a recent study failed to show cognitive decline in young *PSEN1* mutation carriers that were neuropsychologically assessed at least six years prior to clinical onset (Ringman et al., 2005).

It is unknown yet whether cognitively healthy young individuals bearing causative mutations on the *PSEN1* gene do exhibit abnormal brain activity under cognitive challenge and abnormal structural changes 25 years prior to the clinical onset of AD. According to the progression of neuropathological deposits, the hippocampus and surrounding structures (entorhinal and perirhinal cortices) may exhibit reduced volumes before the clinical manifestation of AD (Fox et al., 1996b; Fox et al., 1996a; Kaye et al., 1997; Reiman et al., 1998). It was found in middle-aged and elderly individuals that genetic mutations do influence the degree and regional pattern of atrophy (Gregory et al., 2005). The majority of cases had greater medial temporal atrophy than cases with sporadic disease, suggesting that abnormalities affecting Aß metabolism selectively increased hippocampal degeneration. However, to our knowledge there are no reports in the literature about morphological changes in young *PSEN1* mutation carriers. Because the genetic defect in FAD is present since birth it is conceivable that early neuropathological deposits might induce subtle changes on neurophysiologic function and brain metabolism decades before the clinical onset of the disease.

Here we combined genetics, neuroimaging, and quantitative neuropsychological methods to establish the earliest detectable changes in cognition, brain structure and memory-related activation levels in carriers of the *PSEN1* mutation (C410Y). The mutation carriers and their relatives, who do not carry the pathogenic genes, as well as a group of control subjects from healthy families were examined with fMRI while they were performing hippocampus-sensitive episodic memory tasks and hippocampus independent working memory and attentional tasks. According to the 'compensatory hypothesis' (Bondi et al., 2005; Bookheimer et al., 2000) we hypothesized that young carriers of *PSEN1* mutations do exhibit enhanced brain activity patterns during hippocampus-specific memory tasks at 25 years prior to the clinical manifestation of AD, while retaining normal cognitive performance and normal brain volumes.

Materials and Methods

Subjects

We examined five nondemented members of a family with FAD: three young (~20 years) and two middle-aged (~45 years) individuals. Of the five individuals a young man of 20 years and a middle-aged female of 45 years carried the C410Y mutation on the *PSEN1* gene. We also describe neuropsychology data of two index patients of the family that were clinically followed up at our ward. For anonymity reasons the family pedigree and any familial degrees of relationship are not reported in this article. As for further reading, the nondemented family members are denoted with a Y or an M determining their age group (young or middle-aged). The index patients are denoted with an I. In case that they are *PSEN1* mutation carriers, the abbreviation of the mutation is indicated. The control group consisted of 21 young and healthy subjects. They were matched according to the young mutation carrier's age, years of education and APOE genotype (see Table 1 for demographics of the family members and the control group). All subjects, except for the index patients, did not suffer from current psychiatric or neurological problems and denied to take drugs or medication. Their anatomical T1-weighted MRI scans showed normal brain structures as rated by a neurologist (J.S.). All subjects gave written informed consent to participate in the study after the nature and possible consequences of the study had been explained. The experiments were approved by the ethics committee of the Kanton Zurich.

Table 1 Demographics

	I1 C410Y	*I2 C410Y*	Y1 C410Y	Y2	Y3	M1 C410Y	M2	Controls (n = 21)
Age	54	52	20	20	23	45	52	22.2 ± 1.75
Years of education	15	16	13	12	15	13	9	14.13 ± 1.39
Sex	m	m	m	f	f	f	f	f (n = 15), m (n = 6)
APOE	ε2/ε3	ε2/ε3	ε2/ε3	ε2/ε3	ε2/ε4	ε2/ε3	ε2/ε3	ε2/ε3 (n = 11), ε3/ε3 (n = 10)
Age of onset	49	48	--	---	---	---	---	---

Values: means ± SD, I: indexpatient, Y: young, M: middle-aged, C410Y: mutation on the PSEN1 gene, APOE: apolipoprotein, m: male, f: female

Genetic analyses

Genomic DNA was extracted from whole blood. Mutation screening was performed by direct sequencing of both strands of PCR-amplified coding exons of PSEN1 (exon 2-12), PSEN2 (exon 3-12), exons 16 and 17 of APP, and the single coding exon of PRNP in one index patient. In all other members of the family only the mutation site was verified by sequencing the exon. In brief, amplification was done by a universal "touch down" protocol, using primers as described earlier (available on request) (Finckh et al. 1998). Purified PCR – products were sequenced by cycle sequencing, using fluorescent dye dideoxy terminators (ABI PRISM® BigDye™ Terminators v 3.0 Cycle Sequencing Kit) and analysis was performed on a (ABI PRISM® 310 Genetic Analyzer). APOE genotypes were assessed using the LightCyclerTM instrument (Roche Diagnostics Corporation) (Bernard et al. 1999).

Experimental procedure

All subjects, except the indexpatients, underwent two fMRI experiments, one on episodic memory and the other one on working memory (please see Figure 1 of the APOE study for an illustration of the study design). Trials were blocked in each fMRI experiment. Subjects practiced all fMRI tasks prior to scanning. The same stimuli were used for all subjects to reduce inter-subject variance caused by stimulus-generated effects. Responses were collected with a response box that subjects held in their dominant hand.

fMRI Experiment on episodic memory – Encoding. We presented 16 face-profession pairs for associative learning, 16 faces for single face learning and 24 head contours without physiognomy in the visual baseline condition. The face-profession pairs and the single faces were learned over three consecutive learning runs (three separate fMRI time series). The instruction for associative learning of the face-profession pairs was to imagine the presented person acting in a scene of the written profession. Subjects answered by button press whether they found it easy or hard to imagine a scene. The imagination of a scene automatically leads to the establishment of semantic person-occupation associations and activates the

hippocampal formation (Degonda et al., 2005). Importantly, subjects were requested to imagine the same scene for a given face-profession pair during runs 2 and 3 as during run 1. The instruction for the learning of single faces was to decide whether a face was pleasant or unpleasant. This task yields a semantic encoding of faces. The visual baseline task was to decide whether the area of the left or right ear was larger. The sequence of conditions within the fMRI time-series was counterbalanced across subjects. Each learning condition consisted of four blocks. A block contained four trials of 6 s each. The baseline condition consisted also of four blocks. Here, a block contained six trials of 4 s each. Consequently, each task block took 24 s. An instruction slide announced each task block.

fMRI Experiment on episodic memory – Retrieval. We applied a single fMRI time-series for the retrieval of the previously learned face-profession associations and faces. This time-series included an associative retrieval condition, a face recognition condition, a new faces (distracter faces) detection condition and the same visual baseline condition that was used for the encoding time-series. For the retrieval of the associations, the previously presented faces were shown again (without the professions) as retrieval cues with the instruction to recall each person's occupation and to indicate the superordinate professional category by button press: academic or workman. For face recognition/new face detection, old (studied) and new faces were presented with the instruction to indicate by button press whether a face was fully recollected, appeared just familiar or was completely new (Tulving, 1985). The sequence of conditions within the fMRI time-series was counterbalanced across subjects. All conditions, except for the visual baseline condition (see above), consisted of four blocks, each block including four trials of 6 s each. All task blocks took 24 s and were announced by an instruction slide.

fMRI Experiment on working memory. The experiment included one fMRI time-series with a 2-back task for the assessment of working memory and a baseline task ('x-target') for the assessment of concentration. The 2-back task required subjects to respond to a letter repeat with one intervening letter (e.g. **S** – f – **s** – g). The 'x-target' task required subjects to respond to the occurrence of the letter 'x'.

Each task was given in five blocks of 26 s each. Blocks were announced by an instruction slide. Stimuli were 50 upper- or lowercase letters typed in black on a white background. Thirteen upper- or lowercase letters were presented per block for the duration of 2 s each.

Data acquisition

MR measurements were performed on a 3T Philips Intera whole body MR scanner equipped with an eight-channel Philips SENSE head coil. Functional data were obtained from 32 transverse slices parallel to the AC-PC plane covering the whole brain with a measured spatial resolution of 2.8 x 2.8 x 4 mm^3 (acquisition matrix 80 x 80) and a reconstructed resolution of 1.7 x 1.7 x 4 mm^3. Data were acquired using a SENSE-sshEPI (Schmidt et al., 2005) sequence with an acceleration factor of R = 2.0. Other scan parameters were TE = 35 ms, TR = 3000 ms, $\theta = 82°$. A standard 3D T1-weighted scan was obtained for anatomical reference with a measured spatial resolution of 1 x 1 x 1.5 mm^3 (acquisition matrix 224 x 224) and a reconstructed resolution of 0.9 x 0.9 x 0.8 mm^3, TE = 2.3 ms, TR = 20 ms, $\theta = 20°$. A 2D T1-weighted inversion-recovery anatomical scan, oriented perpendicularly to the long axis of the hippocampus, was obtained for hippocampal and parahippocampal volumetry over 33-39 slices with a measured spatial resolution of 0.5 x 0.6 x 1.5 mm^3 (acquisition matrix 400 x 320) and a reconstructed spatial resolution of 0.4 x 0.4 x 1.5 mm^3, TE = 15 ms, TR = 4200 ms, $\theta = 20°$, IR delay 400 ms, no interslice gaps.

Analysis of functional MRI data

Image pre- and postprocessing and the statistical analyses were performed with SPM2 (http://www.fil.ion.ucl.ac.uk/spm). Standard preprocessing procedures were applied in two ways: once with realignment, spatial normalization and spatial smoothing (8 mm) (Friston et al., 1995a) for a random effects analysis (RFX), and once with realignment and smoothing (4mm) - no spatial normalization - for an additional region-of-interest (ROI) analysis of each subject. On the single subject level, data were analyzed according to the fixed effects model (SPM2). The six head movement parameters were included in the model as confounding factors. Data were high-pass filtered with a specific filter-value for each fMRI time-series. This value was determined according to '2*SOA*TR'. Contrasts for each subject were calculated by subtracting the baseline condition from the experimental condition. On the second level, within-subject contrasts were entered into a RFX analysis (Two sample T-tests, SPM2) which accounts for variance between subjects (Friston et al., 1995b). It is possible in SPM to compare a single subject with a group of controls. The two-sample t-test implemented with that option in SPM takes the control groups' variance as an estimate for the single subject. Thresholds were set at a $p < 0.001$ level, uncorrected for multiple comparisons, with an extend threshold of 15 voxels; exceptions ($p < 0.005$, 3 voxels extend) were made for the region of interest, i.e., the hippocampus, and are indicated where applicable.

For the purpose of a more accurate statistical analysis in the hippocampus a Region-of-interest (ROI) analysis was performed using the MarsBar toolbox in SPM2 (Brett et al., 2002). Both hippocampi were manually delineated on the 3D-T1-weighted structural MRI images of each subject. Mean contrast values for each subject and condition were extracted for each ROI. The mean contrast values of each family member were then separately compared to the 7th (P7) and 93rd (P93) percentile of the control group's mean contrast values. The P7 and the P93 correspond to +/- 1.5 SDs.

Analysis of anatomical MRI data

Based on the 3D-T1-weighted structural MRI images which covered the whole brain, volumes of the total gray and white matter were computed with SPM2. Images were first normalized into the MNI T1 template using a standard bounding box and then segmented into gray matter, white matter and cerebrospinal fluid. Standardized gray and white matter volumes were then multiplied by the determinant of the linear transformation matrix to obtain gray and white matter volumes in cm^3. Based on the 2D-T1-weighted high resolution structural MRI images, two independent raters (A.B. and H.M.) manually delineated the hippocampal formation (Henke et al., 1999a) (CA regions, dentate gyrus and subiculum, excluding the fimbria) and the parahippocampal gyrus using the software Pmod (http://www.pmod.com.). Cerebrospinal fluid was carefully excluded which resulted in conservative volume estimates. Raters relied on descriptions of anatomical landmarks and subdivisions of the MTL as described by Insausti et al. (1998) and Duvernoy (1998). Inter-rater reliabilities ranged between r = 0.8 and 0.98. Each family member's volumes were compared to the P7 and P93 of the control group's volumes. The male mutation carrier was separately compared to a control group consisting of 9 males (age mean 21.1, SD 1.7), while the four female family members were compared to a female control group (n = 13; age mean 22, SD 1.8).

Neuropsychology

The index patients were examined with standardized dementia screening tests like the Mini Mental State Examination (MMSE) (Folstein et al., 1975), the test battery of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Morris et al., 1989) and the Alzheimer's Disease Assessment Scale - Cognitive Subscale (ADAS-cog) (Rosen et al., 1984). Additional tests were applied for the measurement of executive functions, language abilities, spatial cognition, semantic memory, perception, and practical acting.

In the nondemented family members, memory functions were assessed with the Wechsler Memory Scale Revised (WMS-R) (Härting et al., 2000) in German. Intelligence was measured with the Hamburg Wechsler Intelligence Test (HAWIE) (Tewes, 1991). Spatial cognition was tested with the Luria Mental Rotation Test (Christensen, 1979). Executive functions were assessed with a verbal (S-Words) and a nonverbal (5-Point) fluency task (Regard et al., 1982), with the Kramer Card Sorting Test (Kramer, 1970), which measures concept finding and shifting abilities, and with the Stroop Test (Stroop, 1935), which measures the suppression of interference. The two middle-aged family members were additionally examined with the MMSE and the CERAD battery. Test scores of each family member were compared to the P7 and P93 of the control group's scores. Furthermore, the subjects' scores in the WMS-R and HAWIE subtests were compared to an age-matched normative population.

Results

Neuropsychology

Follow up - indexpatients

The clinical follow up of the index patients started in the years 2000/2001 at our ward, and is still running.

Patient *I1 C410Y* had his first visit at our outpatient ward at the age of 51. He was disoriented to time and place and presented with pronounced deficits (> 2 SDs) in the learning, retrieval and recognition of verbal and figural material. We also found impairments in executive functions and semantic memory (factual knowledge). In the next two years the patient was seen another three times for neuropsychological assessment. The episodic memory deficits progressed until almost complete memory loss of recent events. Also, orientation and executive functions got worse and the patient started to show signs of apraxia and deficits in working memory and language.

Patient *I2 C410Y* first presented at our outpatient ward at the age of 50 years. He was examined five times during a period of 4 years. At the initial visit he presented with a selective impairment of retrieval of previously learned verbal and figural material (> 2 SDs). Learning and recognition of this verbal and figural material were slightly reduced. Executive functions were slightly reduced as well. Memory deterioration then progressed during the next four years until he almost completely lost memory for recent events. The patient exhibited apraxia and more pronounced deficits in executive functions, working memory, semantic

memory, spatial thinking and visuoconstruction. However, language abilities remained preserved.

Young subjects

The young mutation carrier *Y1 C410Y* fell below the control groups 7th percentile in the indices for visual memory and delayed memory of the WMS-R (Fig. 1). The subtests that mainly contributed towards the low ranks in both indices were the visual paired associations (VPA) learning and recall tasks. This hints towards a selective impairment of visual memory capacity in the young mutation carrier. When compared to a normative age-matched population, performance scores in the visual paired associations tasks were more than two SDs below the population mean (Score VPA1; *Y1 C410Y*: 10, Controls: mean: 14.10, SD: 3.14, score VPA2; *Y1 C410Y*: 4, controls mean: 5.48, SD: 0.78). According to (Petersen et al., 2001) these deficits in visual memory would be clinically diagnosed as a mild cognitive impairment (MCI), a transitional state between the cognitive changes associated with normal aging and with AD. In other words, persons with MCI experience memory loss to a greater extent than one would expect for their age. The selectivity of the visual memory impairment is underscored by the fact that in all other neuropsychological tests subject *Y1 C410Y* performed within the P7/P93 range. Furthermore, this subject impressed with a high total intelligence quotient, that nearly reached the control group's P93 (Fig. 1). For an overview of the neuropsychology scores of all five family members please see supplementary table S1.

Subject's *Y2* scores in the memory and IQ indices and in all the other neuropsychological tests were located within or above the P7/P93 range of the control group's performance (Fig. 1). This subject can therefore be regarded as a cognitively healthy young study participant.

Subject *Y3* showed deficits on a broad range of cognitive functions. All memory indices, the total IQ score, the verbal IQ score, and verbal fluency ranged below the 7th percentile of the control group (Fig. 1). This reduced general cognition is probably standing for a naturally low ability which is unrelated to FAD.

Middle-aged subjects

The 45-years old mutation carrier *M1 C410Y* fell off the control groups 7th percentile in all memory indices of the WMS-R but retained total IQ and all further performance scores within the P7/P93 range (Fig. 1). The performance scores in the memory subtests were below two SDs of a normative age-matched control group's mean (Table S1). This subject presents with

progressed selective memory impairments and can therefore be clinically diagnosed with MCI (Petersen et al., 2001), which eventually will develop into an Alzheimer's dementia in the near future.

Subject *M2* (52 years old) scored below the P7/P93 range only in the total IQ and the verbal IQ index (Fig. 1). She can therefore be regarded as a cognitively healthy middle-aged study subject with a naturally low cognitive level.

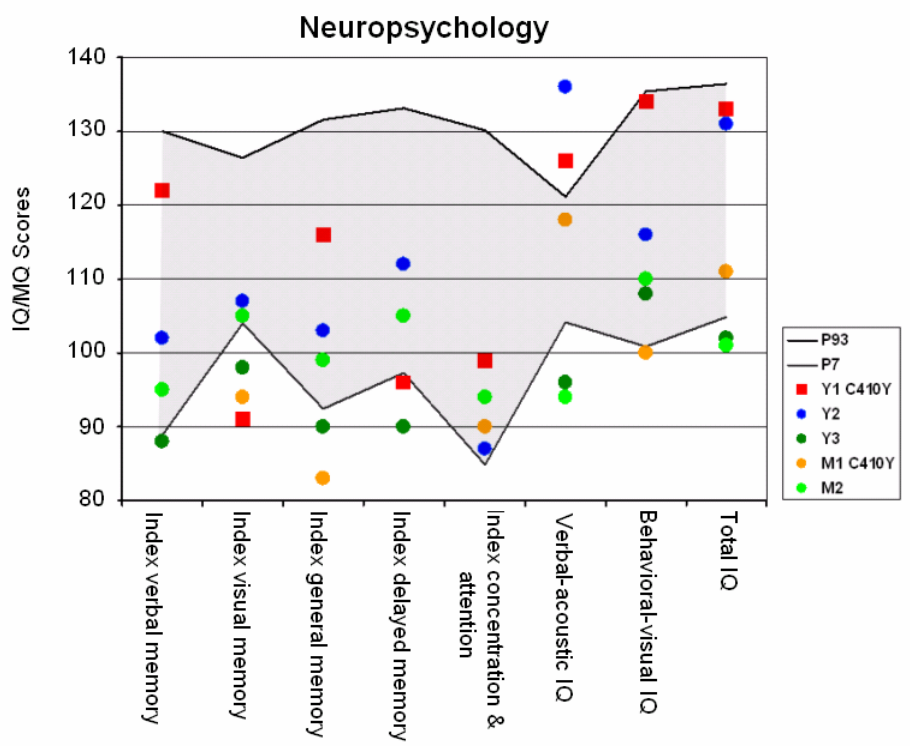

Fig. 1. Neuropsychology data. Shown are the performance scores of the five family members in the indices of the Wechsler Memory Scale revised (WMS-R) and the Hamburg Adult Wechsler Intelligence Scale (HAWIE). Family members are denoted by a colored symbol (see legend). The grey area reflects the control group's performance between the 7th and the 93rd percentile. IQ, intelligence quotient; MQ, memory quotient.

Behavioral results – fMRI memory tasks

The *young subjects* performed within the P7/P93 range of the control group's performance during the associative and the single face learning/retrieval tasks and during the working memory and the attentional tasks (Table 2).

Each and every young subjects' number of 'easy' (to imagine) answers during associative learning increased over learning runs, indicating effective learning over runs. Likewise, their 'pleasant' answers increased over runs during single face learning, indicating that repeatedly presented faces got more familiar and consequently were judged more pleasant.

In contrast to the young family members, both of the two *middle-aged subjects* decreased the number of their 'easy' answers over runs during associative learning, with the middle-aged mutation carrier *M1 C410Y* having the smallest amount of 'easy' answers of all familiy members over the three runs (Σ 'easy' all runs; *Y1 C410Y*: 32, *Y2*: 31, *Y3*: 28, *E1 C410Y*: 25, *M2*: 31, see Table 2). However, the number of 'pleasant' answers during single face learning increased over runs for each of the two middle-aged family members, and ranged within P7/P93 of the control group. This indicates that imagining a scene during associative learning was more difficult for the middle-aged subjects than learning a single face. Interestingly, the middle-aged subjects fell below the control group's P7/93 range only for the single face retrieval task and for the novel face detection task, but not for the associative retrieval task. The middle-aged mutation carrier *M1 C410Y* achieved the smallest amount of 'remember' and 'know' answers during single face retrieval as compared to the control group and to the other family members (Σ 'remember' & 'know'; *Y1 C410Y*: 15, *Y2*: 14, *Y3*: 12, *E1 C410Y*: 3, *M2*: 8, see Table 2). The other middle-aged subject *M2* had also only a few 'remember' answers, but in her case, she compensated her low retrieval performance from episodic memory with a larger amount of 'know' (semantic memory) answers. Both middle-aged subjects unreliably recognized new faces, falling clearly below the control group's P7/P93 range. Subject *M2* even recognized only one new face out of 16. This bad performance was mainly due to a lot of false positive answers during the novel face detection task. Subject *M2* also performed badly in the 2-back task, again because of a large amount of false positives. This might indicate an attentional problem of this subject during the working memory and single faces tasks. However, the decreased performance of subject *E1 C410Y* appears only in episodic memory tasks, and therefore reliably mirrors her deficits in the neuropsychological examination.

Table 2 Performance fMRI memory tasks

	Y1 C410Y	Y2	Y3	M1 C410Y	M2	Controls*
Associative learning						
# easy (run1)	9	9	9	9	11	8.76 ± 2.28
# easy (run2)	10	12	9	9	10	9.57 ± 2.64
# easy (run3)	13	10	10	7	10	10.67 ± 2.35
# easy (run1 - run3)	-1	-4	-1	**2**	**1**	-1.9 ± 2.07
Single face learning						
# pleasant (run1)	8	8	5	7	6	7 ± 2.21
# pleasant (run2)	8	8	4	8	6	7 ± 2.72
# pleasant (run3)	9	9	5	9	7	7.29 ± 2.49
# pleasant (run1 - run3)	-1	-1	0	-2	-1	-0.29 ± 1.49
Associative retrieval						
# correct	11	11	9	11	11	11.9 ± 2.2
Single face retrieval						
# correct remember *(hits - false alarms)*	14	11	7	**2**	**2**	10.25 ± 3.75
# correct know *(hits - false alarms)*	1	3	5	1	6	2.95 ± 3.55
Novel face detection						
# correct *(hits - false alarms)*	16	14	12	**6**	**1**	12.86 ± 3.44
Working memory						
2-back # correct *(hits - false alarms)*	11	10	10	8	**3**	8.85 ± 2.89
Attention						
x-target *(hits - false alarms)*	13	13	13	13	12	12.95 ± 0.22

* means ± SD

Functional imaging results

Young subjects

As compared to controls, the young mutation carrier *Y1 C410Y* showed enhanced brain activity distributed over many brain regions during learning and retrieval, novelty detection, and during the working memory experiment (see Supplementary Table S2 for locations of brain activity). Importantly, reversing the contrasts (i.e. comparing the control group with subject *Y1 C410Y*) yielded some brain areas (left superior frontal gyrus, left cingulate and bilateral superior temporal gyrus) with enhanced activity for the working memory task but not for learning/retrieval tasks nor for novelty detection. The most prominent activity enhancement of subject *Y1 C410Y* versus the control group was found in a left-lateralized transcortical network including the hippocampus during associative learning (Table 3) and in a left fronto-temporo-parietal network during associative retrieval (Table 4).

A ROI analysis confirmed that the young mutation carrier's hippocampal activity during associative learning ranged above the control group's 93rd percentile of the control group's activity. This activity enhancement could be attributed to the middle portion of the left hippocampus (Figure 2). In all the other contrasts, hippocampal activity ranged within the control group's P7/P93 (Figures 2 & 3).

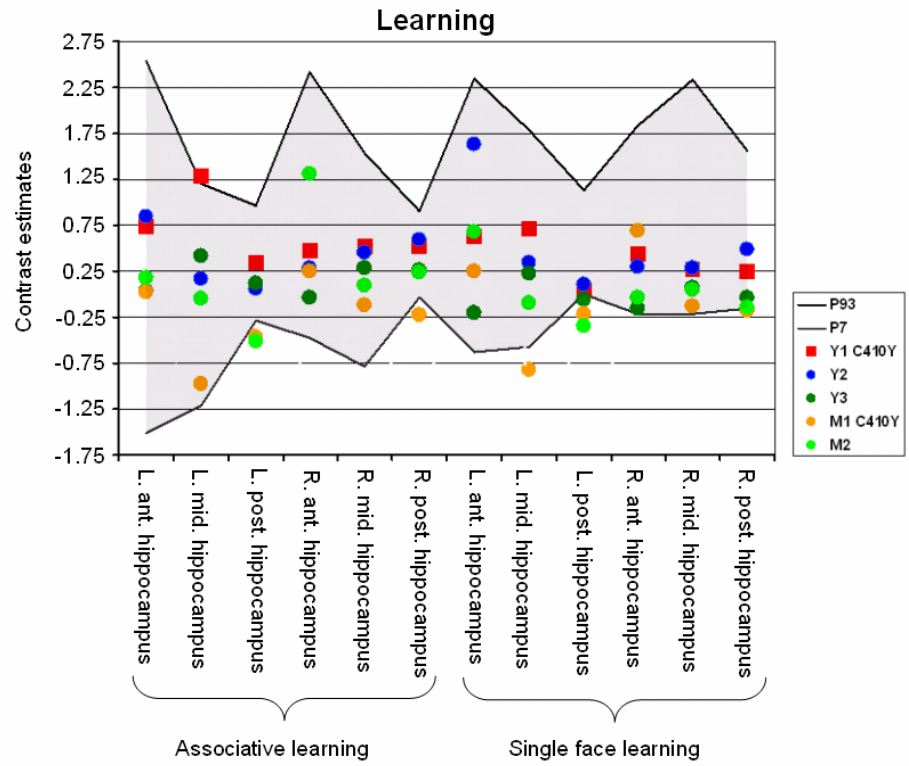

Fig. 2. ROI analysis data - learning tasks. Shown are the family members' contrast estimates in the left and right hippocampal subdivisions (i.e. anterior, middle, posterior) during associative and single face learning. Family members are denoted by a colored symbol (see legend).The grey area reflects the control group's contrast estimates between the 7th and the 93rd percentile.

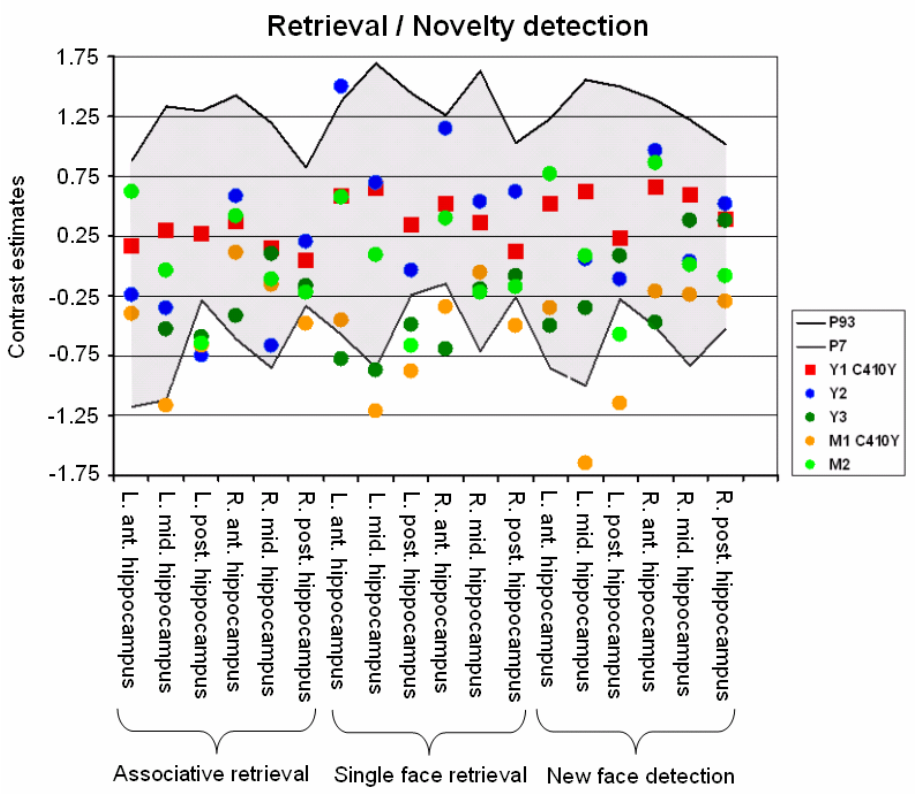

Fig. 3. ROI analysis data - retrieval and new face detection tasks. Shown are the family members' contrast estimates in the left and right hippocampal subdivisions (i.e. anterior, middle, posterior) during associative and single face retrieval, and new face detection. Family members are denoted by a colored symbol (see legend).The grey area reflects the control group's contrast estimates between the 7th and the 93rd percentile.

To gain further evidence that the enhanced activity in subject *Y1 C410Y* was related to the *PSEN1* gene mutation, we randomly selected 6 controls subjects and compared each of them with the remaining 20 subjects of the control group. As shown in Figure 4 only subject R2 exhibited enhanced activity compared to the controls during associative learning (R2: right superior frontal gyrus, left superior occipital gyrus, left basal ganglia and cerebellum; R5: artefactual activation of white matter). A similar outcome is shown for the associative retrieval contrast where again only two subjects, R2 and R4, exhibited two spots of enhanced activity as did controls (R2: left middle frontal gyrus, left cerebellum; R4: left middle and superior frontal gyrus). However, the activity increases in these control subjects were of a smaller cluster size and only present in very few brain regions as compared to the young mutation carrier.

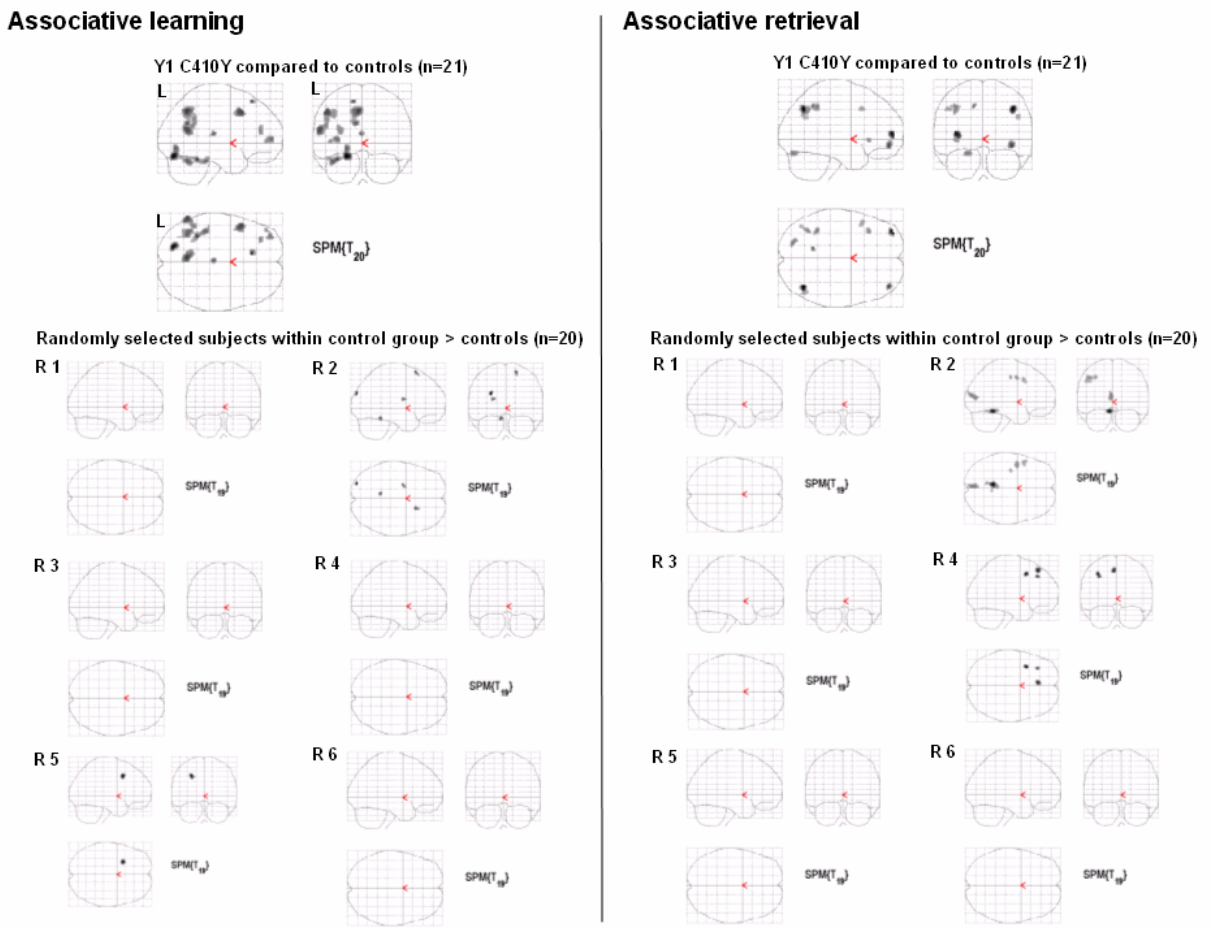

Fig. 4. Brain activity enhancement in the young mutation carrier during associative learning and retrieval. Upper panel: Activity differences between the young mutation carrier and the control group during associative learning and retrieval are depicted on a glass brain. Lower panel: Six randomly selected control subjects were compared to the control group. Activity differences are depicted on a glass brain. Note that subject Y1 C410Y reliably shows enhanced activity in various brain areas as compared to controls during associative learning and retrieval while the six randomly selected controls subjects show only marginal differences or none at all.

Subject *Y2* also showed a few neocortical brain areas with enhanced brain activity during learning and retrieval, novelty detection and during the working memory task as compared to the control group (Supplementary Table S3). Reversing the contrasts yielded some regions of stronger activity in the control group as well, but only during the associative retrieval task (Table 4) and the new face detection task (Supplementary Table S3).

The ROI analysis revealed that subject's *Y2* left posterior hippocampal activity ranged below the 7th percentile of the control group's activity during associative retrieval, while hippocampal activity in the left anterior hippocampus was above the 93rd percentile during single face retrieval.

Enhanced brain activity during learning/retrieval, novelty detection and working memory was also found in subject *Y3* as compared to the control group. Again, similarly to subject *Y2*, enhanced brain activity occurred only in a few neocortical brain areas (Supplementary Table S4). By reversing the contrasts we could see that the control group exhibited enhanced brain activity in various areas, predominantly in medial temporal lobe structures (MTL) during associative retrieval (Table 4), face recognition and new face detection (Supplementary Table S4). Differences in activity of MTL structures were replicated in the ROI analysis. Here subject *Y3* fell below P7 in the left posterior hippocampus during associative retrieval and in the left anterior, middle and posterior hippocampus and the right anterior hippocampus during single face retrieval. In the new face detection task hippocampal activity ranged near the control's 7th percentile (Figures 2 & 3).

Middle-aged subjects

The middle-aged mutation carrier *E1 C410Y* showed almost no brain areas with enhanced activity compared to controls, except in the right middle frontal gyrus during single face learning, the right orbital gyrus during associative retrieval and some fronto-parietal areas during the working memory task (Supplementary Table S5). Reversing the contrasts revealed that the control groups' brain activity was stronger in MTL structures and a memory-related neocortical network as compared to *M1 C410Y* in all contrasts, except during working memory. Again, these differences of activity strength between the family member and controls became mainly apparent during associative learning and retrieval (Tables 3 & 4).

The lower activity level of the middle-aged mutation carrier was also apparent in the ROI analysis. Here, activity in various hippocampal subdivisions strikingly fell below the control group's 7th percentile during single face and associative learning/retrieval and during novelty detection (Figures 2 & 3).

Subject *M2* showed enhanced brain activity in a few areas during associative learning and working memory, as compared to the control group (Supplementary Table S6). Reversing the contrasts showed enhanced activity of the control group in the hippocampus and memory-related neocortical areas mainly during associative learning and retrieval (Tables 3 & 4, Supplementary Table S6) but also during face recognition and novelty detection.

The ROI analysis revealed that activity in the left posterior hippocampus fell off P7 during learning/retrieval and during novelty detection in subject *M2* (Figures 2 & 3).

We analyzed repetition effects in each family member during learning by contrasting the first with the third learning run. This analysis did not yield a reliable effect of the *PSEN1* mutation on brain activity underlying repeated learning. Therefore this data is not shown.

Taken together, these results show that the young mutation carrier exhibited enhanced activity in memory-related neural structures compared to his young family relatives and compared to the control group. This enhancement of brain activity appears in all contrasts, though predominantly during associative learning and retrieval, a measure of episodic memory (Table 4). This pattern of activation stands in clear contrast to the other two young family members in whome only a few brain areas of enhanced activity were detected. Furthermore both young mutation noncarriers also exhibited areas of decreased activity, while subject *Y1 C410Y* almost exclusively exhibited areas of enhanced activity.

Nearly the opposite pattern was seen in the middle-aged subjects. Here, the mutation carrier exhibited almost only areas of decreased memory-related activity during learning and retrieval. In comparison the non-carrier showed only few areas of decreased activity, but also some areas of enhanced activity.

Structural imaging results

Manual volume measurements of both hippocampi and both parahippocampal gyri revealed comparable volumes between the family subjects and the respective control groups (Supplementary Table S7). Automated whole brain grey and white matter segmentation showed less (below the control group's 7th percentile) grey matter in subjects *Y1 C410Y* and *Y3*. However, this differences in grey matter volumes are not explainable by grey matter loss but rather by small head sizes of these two subjects, as was confirmed by their head transformation matrices (data not shown) resulting after normalization in SPM2.

Table 3 Brain activation differences between the family subjects and the control group during associative learning

Brain Region	Left/Right	MNI Coordinates (mm) x	y	z	BA	kE	t
Two sample T-Test							
Associative learning							
Y1 C410Y > Controls							
Hippocampus	L	-38	-34	-6		7	3.44[a]
Fusiform gyrus	L	-22	-78	-18	19	147	7.31
	L	-34	-46	-22	37	210	6.02
Superior frontal gyrus	L	-12	30	58	8	20	6.3
Middle frontal gyrus	L	-50	16	42	8	112	5.92
	L	-34	56	4	10	61	5.02
	L	-30	42	16	46	47	4.68
Middle temporal gyrus	L	-60	-58	16	37	210	5.78
Inferior parietal lobule	L	-38	-60	38	7	57	4.44
Precuneus	L	-6	-60	44	7	265	5.98
Y2 > Controls							
Superior temporal gyrus	R	52	16	-12	38	20	3.8
Insula	R	40	-14	2		16	4.12
Precentral gyrus	L	-54	4	20	6	15	4.18
Y3 > Controls							
Fusiform gyrus	R	40	-50	-20	37	19	4.69
(Fusiform gyrus/Cerebellur	L	-46	-48	-30	37	19	4.66
M1 C410Y > Controls							
no significant differences							
E2 > Controls							
Superior frontal gyrus	L	-18	-14	60	6	33	4.69
Superior temporal gyrus	R	46	-28	4	22	44	5.36
Fusiform gyrus	R	-20	-74	-8	19	32	5.43

Brain Region	Left/Right	MNI Coordinates (mm) x	y	z	BA	kE	t
Associative learning							
Controls > Y1 C410Y							
no significant differences							
Controls > Y2							
no significant differences							
Controls > Y3							
Superior temporal gyrus	L	-58	-24	10	22	18	4.13
Postcentral gyrus	L	-42	-26	64	1	53	8.34
Controls > M1 C410Y							
Hippocampus	R	26	-10	-22		17	4.56
	R	26	-30	-6		5	3.98[a]
Amygdala/Hippocampus	L	-32	-8	-26		11	4.74[a]
Middle frontal gyrus	L	-24	60	-2	10	72	7.45
	R	24	60	8	10	31	4.92
Superior temporal gyrus	L	-56	-2	-4	22	101	6.16
	L	-50	-20	4	22	92	6.1
	R	52	-8	-4	22	38	4.29
Cingulate gyrus	R	2	38	6	24	35	3.58
Fusiform gyrus	L	-32	-36	-30	36	26	4.65
Controls > E2							
Hippocampus	R	36	-28	-14		48	5.8
	R	22	-28	-12		5	4.33[a]
Parahippocampal gyrus	L	-14	-44	-4	30	624	7.77
Superior temporal gyrus	R	44	2	-24	22	23	5.2

t: values of peaks within significantly activated clusters of voxels, kE: cluster size (in voxels), BA: Brodman area, a $p < 0.005$

Table 4 Brain activation differences between the family subjects and the control group during associative retrieval

Brain Region	Left/Right	MNI Coordinates (mm) x	y	z	BA	kE	t
Two sample T-Test							
Associative retrieval							
Y1 C410Y > Controls							
Middle frontal gyrus	L	-38	56	8	10	49	6.08
	L	40	52	-10	11/10	47	5.23
	L	-50	16	42	8	23	3.95
Inferior frontal gyrus	L	-38	24	-2	47	19	4.22
Fusiform gyrus	L	-22	-78	-20	19	35	4.41
Superior parietal lobule	R	40	-64	40	7	61	6.08
	L	-38	-46	44	7	36	4.34
Inferior parietal lobule	L	-38	-60	40	7	41	4.24
Supramarginal gyrus	R	50	-64	32	40	18	3.91
Y2 > Controls							
Inferior frontal gyrus	R	44	34	-6	47	44	5.6
Y3 > Controls							
Fusiform gyrus	R	40	-52	-24	37	76	7.11
	R	34	-70	-20	19	36	4.28
	L	-44	-48	-26	37	17	4.72
M1 C410Y > Controls							
Orbital gyrus	R	18	54	-10	11	22	6.07
M2 > Controls							
no significant differences							

Brain Region	Left/Right	MNI Coordinates (mm) x	y	z	BA	kE	t
Associative retrieval							
Controls > Y1 C410Y							
no significant differences							
Controls > Y2							
Superior frontal gyrus	L	-2	64	16	10	33	4.62
Cingulate gyrus	L	-8	-58	26	31	37	4.6
	L	-6	46	6	32	43	4.49
Controls > Y3							
Hippocampus/Rhinal cortex	L	-12	-36	-2		15	4.21
Hippocampus	R	26	-28	-10		3	3.08[a]
Rhinal Cortex	L	-22	-6	-32		15	5.02
	L	-22	-22	-18		15	3.18[a]
Medial frontal gyrus	R	2	58	-10	10	15	5.02
Cingulate gyrus	L	-8	-48	10	29	147	5.11
Controls > M1 C410Y							
Hippocampus	L	-22	-30	-10		10	4.07[a]
	R	24	-10	-20		25	3.68[a]
Parahippocampal gyrus	R	30	-32	-14		39	3.81[a]
Entorhinal cortex	L	-16	-4	-32		19	5.43
Superior temporal gyrus	L	-52	-2	-6	22	51	5.38
	R	62	-8	4	22	18	4.36
Controls > M2							
Hippocampus	R	34	-30	-12		95	5.17
	L	-26	-34	-8		14	4.24[a]
Middle frontal gyrus	L	-40	10	54	6	71	4.93
Middle temporal gyrus	R	48	10	-26	21	74	5.27
Cingulate gyrus	L	-6	-46	14	30	17	5.03

t: values of peaks within significantly activated clusters of voxels, kE: cluster size (in voxels), BA: Brodman area, a $p < 0.005$

Discussion

In this study we examined five nondemented members of a family with autosomal dominant FAD to determine preclinical signs of the disease. A young man (20 years) and a middle-aged woman (45 years) carried the C410Y mutation in the *PSEN1* gene. The five subjects underwent fMRI while performing episodic and working memory tasks. Their brain volumes were measured with structural imaging and cognitive status was assessed by applying quantitative neuropsychological methods.

We found that in the young *PSEN1* mutation carrier neuropsychological performance scores were selectively decreased (> 2 SDs) in tasks measuring visual and delayed memory. Such a selective impairment in episodic memory is clinically diagnosed as MCI (Petersen et al., 2001). Although age of onset is around 48 years in this family, memory loss is already detectable 28 years prior to AD diagnosis in this individual case. Because this subject performed very well in all other neuropsychological tests and impressed with high intelligence, we think that the selective drop in visual episodic memory must be related to neuronal dysfunction in the MTL. Selective memory loss was also detected in the middle-aged mutation carrier at age 45. In comparison to the young mutation carrier, her deficits were further progressed and affected both verbal and visual memory. In contrast, one of the young mutation noncarriers showed unspecific broad cognitive deficits that are attributable with high probability to her inherent low intelligence and low general cognitive capacity. Therefore, we think that the selective memory impairments in the young and the middle-aged mutation carrier are specifically related to the *PSEN1* C410Y mutation. In a longitudinal study Fox et al. (1998) found earliest clinical and neuropsychological features of FAD only 4-5 years before individuals at genetic risk for FAD fulfilled criteria for probable AD. Likewise, a recent study failed to discover memory loss decades before the onset of FAD (Ringman et al., 2005). However, frontal-executive dysfunctions have been found in young subjects at risk for frontotemporal dementia due to a pathogenic mutation in the tau gene (FTD-17). These dysfunctions have been attributed to a neurodevelopmental component (Geschwind et al., 2001). The early impairment of episodic memory in our young mutation carrier likely reflects an early dysfunction of MTL structures. According to the neuropathological course of AD (Braak and Braak, 1996; Delacourte et al., 1999; Price et al., 1991) our data suggest that this subject might be suffering from early neuropathological deposits in MTL structures, impacting negatively on episodic memory already some 30 years before fully pronounced AD. The genetic defect in FAD is present since birth, but it is unclear at what point it starts to show effects at the cellular level. In support of early neuropathological changes are studies in Down's syndrome, where deposition of Aß 1-42 has been observed as early as the third decade (Stoltzner et al., 2000; Teller et al., 1996).

The behavioral results in the fMRI experiments did not differentiate the young mutation carrier from the control group or from the young noncarriers of the family. According to the selective visual and delayed memory deficits of the young mutation carrier in the neuropsychological tests one would expect shortcomings during the fMRI retrieval tasks. That was not the case and the mismatch in memory performance between the

neuropsychological tasks and the fMRI experiments might likely be explained through the repetitive nature of our learning tasks, and the learning instruction which required the subjects to recall their imagined scenes in the subsequent runs. Therefore, repetitive learning and the application of a given learning strategy allowed for a deeper encoding of the material, hence increasing memory performance at retrieval. This notion is underscored by the results during repeated associative and single face learning. Here, the number of easy [to imagine a scene] answers increased from run1 to run3 in each and every young family member reflecting a learning process that got facilitated over runs. The same was true for repeated single face learning, where pleasant answers increased over runs, reflecting that faces got more and more familiar, consequently judged more pleasant (mere exposure effect).

Unexpectedly as well, the middle-aged mutation carrier - although diagnosed with MCI according to her neuropsychological outcome - reached a normal performance during associative retrieval. Her number of easy [to imagine a scene] answers decreased from run1 to run3 during associative learning, reflecting that the associative learning task remained cognitively demanding to her in each run. In contrast, retrieval of single faces alone was far below the control group's mean (> 2 SDs) for both 'remember minus false remember' and 'know minus false know' answers, while the latter was mainly due to a high rate of false positives in the new face detection task. These differences in performance between associative retrieval – which is clearly more difficult – and single face retrieval can best be explained by the nature of the tasks and the depth of processing of the presented stimuli at the level of encoding. The repeated imagination of a scene during associative learning, along with the recall and re-encoding processes of the very same scenes in run2 and run3, have allowed for a much deeper encoding of the associations as compared to the single faces alone, which only had to be rated according to their pleasantness. As reflected in the decreased easy [to imagine a scene] answers over runs, learning of associations was a challenging task for the middle-aged mutation carrier. She therefore might have put more effort into learning the associations than single faces, hence boosting her associative retrieval performance.

The fMRI results showed that both mutation carriers exhibited significantly altered patterns of memory-related brain activity compared to the control group and the family members without the mutation. The young mutation carrier consistently exhibited enhanced brain activity in nearly every task. This enhancement was specifically pronounced during associative learning and retrieval, and was present in the hippocampus and many memory-related neocortical areas. The increase in brain activity was not paralleled by significant performance changes between this subject and the controls, neither by differences in brain

volumes. These results are consistent with a compensatory hypothesis where the young mutation carrier appears to require additional cognitive effort to achieve the same level of performance as the control group. It has been demonstrated for a variety of cognitive tasks that increasing task demands are associated with the spatial extend and the magnitude of brain activity in the task-specialized neural network (Bookheimer et al., 2000; Carpenter et al., 1999; Just et al., 1996). These findings generally indicate that the amount of neural activity that a given task engenders is dependent on the computational demand that the task imposes. Interestingly, larger task-related activity enhancements in the MTL and neocortex have been found in elderly nondemented AD-risk allele (APOE4) carriers (Bondi et al., 2005; Bookheimer et al., 2000) and in cases with selective memory impairment (MCI) (Dickerson et al., 2004; Rosano et al., 2005). This activity enhancement was predictive for further cognitive decline (Bookheimer et al., 2000; Dickerson et al., 2004). The most likely explanation for the increased activation in the MTL and neocortical regions is a compensatory response to accumulating AD pathology (Becker et al., 1996; Bookheimer et al., 2000; Grady et al., 2003). This line of argumentation even holds stronger with regard to the brain activity enhancements found in our young *PSEN1* mutation carrier. Neuropathological changes in this man might have taken place since birth and consequently could have had the potential to impact on cognition and normal neuronal functioning already three decades before the clinical onset of AD. We are aware that multiple nonneural factors may confound the interpretation of changes in the hemodynamic fMRI response during activation, such as AD-specific alterations in vascular physiology (Johnson et al., 2000; Mueggler et al., 2002), age- and disease-related changes in neurovascular coupling (Buckner et al., 2000; D'Esposito et al., 2003), and resting hypoperfusion and metabolism in MCI and AD (De Santi et al., 2001; El Fakhri et al., 2003; Johnson et al., 1998). However, because of the young age of the mutation carrier, we don’t think that these factors are responsible for the amplified fMRI signal.

The middle-aged *PSEN1* mutation carrier exhibited almost exclusively decreased activity in the MTL and memory-related areas in all episodic memory tasks. Her low face recognition performance is the most likely cause for the decreased brain activity in that task. It has been shown in several studies, that retrieval success is associated with enhanced activity, while retrieval failure is associated with decreased activity in memory-related brain structures (Daselaar et al., 2003; Maril et al., 2005; McDermott et al., 2000; Meltzer and Constable, 2005). Furthermore, early stage AD patients with cognitive impairments show decreased task-induced brain activity in brain areas mediating the cognitive demands of the task (Buckner et al., 2000; Kato et al., 2001; Small et al., 1999). Decreased activity in the

associative learning and retrieval tasks can not be explained by a lower memory performance, because she achieved comparable levels to the control group in the associative retrieval task. However, according to her selective episodic memory deficits in the neuropsychological examination this subject can be classified as a MCI patient. In opposition to our findings are studies with fMRI in MCI patients that found compensatory task-induced brain activity which was predictive for later cognitive decline (Dickerson et al., 2004; Rosano et al., 2005). An explanation for this mismatch could be a further progressed accumulation of AD pathology in the middle-aged PSEN1 mutation carrier as compared to sporadic forms of AD. FAD-cases suffer from a faster disease progression and are affected by more abundant neuropathological deposits (Gomez-Isla et al., 1997; Lippa et al., 2000; Sakamoto et al., 2002). Therefore it is likely that the decreased memory-related brain activity in our middle-aged mutation carrier is related to neural dysfunction induced by AD-pathology that progressed beyond MTL structures. The aggressive nature of AD pathology in FAD might be responsible for a much more extensive degree of neural dysfunction than in sporadic forms of AD, hence impeding a recruitment of still functioning neural reserves a few years before diagnosis of the disease.

There are several limitations to our study. Since the frequency of FAD cases is very low, we just managed to examine one family with FAD. Only one young mutation carrier of this family agreed to take part in the study. Therefore the conclusions can not be extended to the majority of probable AD cases and must be considered tentative. However, this is the first study to detect preclinical signs of AD on the behavioural and neural level decades before the onset of the disease. To further validate this finding, a follow up of the examined family would be a great gain. Also, additional examinations with imaging techniques measuring metabolic changes would have given additional and valuable insight into what processes are under way some 25 years before the clinical onset of the disease.

Taken together, our results suggest, that cognitive and neural dysfunction is detectable three decades before clinical onset of AD in *PSEN1* C410Y mutation carriers. It is highly likely that these changes are related to accumulating AD pathology. Although past studies failed to detect early preclinical signs in other *PSEN1* missense mutations, this does not impose that our findings are related to the *PSEN1* C410Y mutation only. We propose that in the future fMRI might be a valuable tool for the early detection of AD. In conjunction with other imaging methods and neuropsychological assessment it will allow us to gain insight into the staging of the disease and into the progression from healthy status to the clinical onset, possibly even in the more frequent sporadic forms.

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Supplementary information

Table S1 Neuropsychology

	Y1 C410Y	Y2	Y3	M1 C410Y	M2	Controls
HAWIE						
Total IQ	133	131	102	111	101	122.5 ± 10.18
Behavioral IQ	134	116	108	100	110	119.7 ± 11.78
Verbal IQ	126	136	96	118	94	121.1 ± 10.35
WMS-R						
Index verbal memory	122	102	88	79	95	113.1 ± 13.03
Index visual memory	91	107	98	94	105	117.9 ± 6.77
Index general memory	116	103	90	83	99	116.9 ± 11.66
Index delayed memory	96	112	90	68	105	117.95 ± 9.65
Index concentration & attention	99	87	94	90	94	102.75 ± 12.78
Luria Mental Rotation						
# correct	10	10	10	10	9	9.5 ± 0.69
Kramer Card Sorting						
# correct	6	6	3	4	5	4.85 ± 1.09
Stroop Color Interference						
high interference run (sec)	19	20.9	21.3	27.3	29	21.29 ± 5.39
S-Words Fluency						
# correct	32	41	9	40	25	34.45 ± 7.29
5-Point Nonverbal Fluency						
# correct	38	44	36	30	28	40.15 ± 7.26

Values: means ± SD, sec: seconds, HAWIE: Hamburg adult Wechsler intelligence examination, WMS-R: Wechsler memory scale revised

Table S2 Brain activation differences between subject Y1 C410Y and the control group during the memory tasks

		MNI Coordinates (mm)								MNI Coordinates (mm)						
Brain Region	**Left/Right**	**x**	**y**	**z**	**BA**	**^{k}E**	**t**	**Brain Region**	**Left/Right**	**x**	**y**	**z**	**BA**	**^{k}E**	**t**
Two sample T-Test															
Y1 C410Y > controls								**controls > Y1 C410Y**							
Associative learning								***Associative learning***							
Hippocampus	L	-38	-34	-6		7	3.44a	no significant differences							
Fusiform gyrus	L	-22	-78	-18	19	147	7.31								
	L	-34	-46	-22	37	210	6.02								
Superior frontal gyrus	L	-12	30	58	8	20	6.3								
Middle frontal gyrus	L	-50	16	42	8	112	5.92								
	L	-34	56	4	10	61	5.02								
	L	-30	42	16	46	47	4.68								
Middle temporal gyrus	L	-60	-58	16	37	210	5.78								
Inferior parietal lobule	L	-38	-60	38	7	57	4.44								
Precuneus	L	-6	-60	44	7	265	5.98								
Single face learning								***Single face learning***							
Middle frontal gyrus	R	-44	50	4	10	113	6.24	no significant differences							
	L	-50	16	42	8	103	5.92								
Fusiform gyrus	L	-18	80	-18	19	204	5.95								
Associative retrieval								***Associative retrieval***							
Middle frontal gyrus	L	-38	56	8	10	49	6.08	no significant differences							
	L	40	52	-10	11/10	47	5.23								
	L	-50	16	42	8	23	3.95								
Inferior frontal gyrus	L	-38	24	-2	47	19	4.22								
Fusiform gyrus	L	-22	-78	-20	19	35	4.41								
Superior parietal lobule	R	40	-64	40	7	61	6.08								
	L	-38	-46	44	7	36	4.34								
Inferior parietal lobule	L	-38	-60	40	7	41	4.24								
Supramarginal gyrus	R	50	-64	32	40	18	3.91								
Face recognition								***Face recognition***							
no significant differences								no significant differences							
New face detection								***New face detection***							
Middle frontal gyrus	L	-38	56	6	10	25	4.72	no significant differences							
Superior parietal lobule	R	40	-66	40	7	44	6.19								
	R	14	-58	50	7	33	4.64								
	L	-40	-62	38	7	46	4.23								
Precuneus	L	-10	-56	46	7	28	4.46								
Working memory								***Working memory***							
Inferior frontal gyrus	L	-48	26	4	45	78	4.98	Superior frontal gyrus	R	4	56	24	9	355	6.9
Middle frontal gyrus	L	-44	20	40	8	182	9.76	Cingulate gyrus	R	4	-46	32	31	27	5.04
	L	-42	36	32	9	23	4.52	Superior temporal gyrus	R	48	-8	-4	22	61	5.39
	R	42	34	40	8	75	6.37		L	-50	-62	24	39	96	5.19
	R	28	-4	50	6	43	5.21								
Inferior parietal lobule	L	-50	-32	32	40	157	8.4								
Superior parietal lobule	L	-12	-52	70	7	533	5.54								
	L	-24	-74	54	7	24	5.05								
	R	8	-52	70	7	206	6.74								
	R	34	-80	40	7	17	4.42								
Cingulate gyrus	L	-8	18	32	24	19	6.12								
Middle temporal gyrus	R	52	-24	-12	7	17	4.42								
Superior temporal gyrus	L	-54	-44	14	22	59	4.64								
Cerebellum	L	-2	-50	-30		223	8.23								
	R	10	-78	-28		18	4.06								

t: values of peaks within significantly activated clusters of voxels, kE: cluster size (in voxels), BA: Brodman area, a: $p < 0.005$

Table S3 Brain activation differences between subject Y2 and the control group during the memory tasks

		MNI Coordinates (mm)								MNI Coordinates (mm)						
Brain Region	**Left/Right**	**x**	**y**	**z**	**BA**	**^{k}E**	**t**	**Brain Region**	**Left/Right**	**x**	**y**	**z**	**BA**	**^{k}E**	**t**
Two sample T-Test															
Y2 > Controls								**Controls > Y2**							
Associative learning								***Associative learning***							
Superior temporal gyrus	R	52	16	-12	38	20	3.8	no significant differences							
Insula	R	40	-14	2		16	4.12								
Precentral gyrus	L	-54	4	20	6	15	4.18								
Single face learning								***Single face learning***							
Middle frontal gyrus	L	-44	44	6	46	33	4.73	no significant differences							
Associative retrieval								***Associative retrieval***							
Inferior frontal gyrus	R	44	34	-6	47	44	5.6	Superior frontal gyrus	L	-2	64	16	10	33	4.62
								Cingulate gyrus	L	-8	-58	26	31	37	4.6
									L	-6	46	6	32	43	4.49
Face recognition								***Face recognition***							
no significant differences								no significant differences							
New face detection								***New face detection***							
Inferior frontal gyrus	R	44	36	-6	47	18	4.93	Middle temporal gyrus	R	46	6	-28	21	43	3.88
Working memory								***Working memory***							
Inferior frontal gyrus	R	32	18	-16	47	36	6.04	no significant differences							
Superior frontal gyrus	R	30	-10	64	6	29	5.03								
Insula	L	-32	10	12		16	5.18								
Cerebellum	L	-4	-84	-32		15	4.71								
	R	26	-32	-32		28	4.22								

t: values of peaks within significantly activated clusters of voxels, kE: cluster size (in voxels), BA: Brodman area, a: $p < 0.005$

Table S4 Brain activation differences between subject Y3 and the control group during the memory tasks

		MNI Coordinates (mm)								MNI Coordinates (mm)						
Brain Region	**Left/Right**	**x**	**y**	**z**	**BA**	**kE**	**t**	**Brain Region**	**Left/Right**	**x**	**y**	**z**	**BA**	**kE**	**t**
Two sample T-Test															
Y3 > Controls								**Controls > Y3**							
Associative learning								***Associative learning***							
Fusiform gyrus	R	40	-50	-20	37	19	4.69	Superior temporal gyrus	L	-58	-24	10	22	18	4.13
Fusiform gyrus/Cerebellum	L	-46	-48	-30	37	19	4.66	Postcentral gyrus	L	-42	-26	64	1	53	8.34
Single face learning								***Single face learning***							
Superior frontal gyrus	L	-14	62	24	10	115	5.96	Superior temporal gyrus	L	-60	-24	10	22/42	114	6.41
Fusiform gyrus	R	42	-52	-22	37	41	4.95	Middle frontal gyrus	R	18	24	40	8	27	4.3
Associative retrieval								***Associative retrieval***							
Fusiform gyrus	R	40	-52	-24	37	76	7.11	Hippocampus/Rhinal cortex	L	-12	-36	-2		15	4.21
	R	34	-70	-20	19	36	4.28	Hippocampus	R	26	-28	-10		3	3.08a
	L	-44	-48	-26	37	17	4.72	Rhinal Cortex	L	-22	-6	-32		15	5.02
									L	-22	-22	-18		15	3.18a
								Medial frontal gyrus	R	2	58	-10	10	15	5.02
								Cingulate gyrus	L	-8	-48	10	29	147	5.11
Face recognition								***Face recognition***							
Fusiform gyrus	R	40	-50	-2	37	208	8.09	Rhinal cortex	L	-22	-16	-30		3	3.01a
	L	-44	-48	-26	37	49	5.18	Medial frontal gyrus	R	2	58	-10	10	74	4.72
Occipital gyrus	L	-40	-84	-12	19	53	5.69								
New face detection								***New face detection***							
Fusiform gyrus	R	42	-50	-22	37	271	8.62	Rhinal cortex	L	-22	-16	-30		8	3.31
	L	-44	-48	-26	37	97	5.82	Medial frontal gyrus	R	2	58	-12	10	17	4.23
Working memory								***Working memory***							
Middle frontal gyrus	R	44	52	6	10	367	7.83	no significant differences							
	R	34	10	44	6	96	5.6								
Superior parietal lobule	R	26	-54	52	7	232	7.76								
	L	-40	-56	54	7	228	6.18								
Inferior temporal gyrus	L	-56	-64	-16	37	179	5.78								
	R	54	-60	-16	37	187	5.31								
Fusiform gyrus/Cerebellum	L	-14	-84	-26	19	380	7.74								
Cerebellum	R	10	-86	-30		112	5.4								

t: values of peaks within significantly activated clusters of voxels, kE: cluster size (in voxels), BA: Brodman area, a: $p < 0.005$

Table S5 Brain activation differences between subject M1 C410Y and the control group during the memory tasks

	MNI Coordinates (mm)								MNI Coordinates (mm)							
Brain Region	**Left/Right**	**x**	**y**	**z**	**BA**	**^{k}E**	**t**		**Brain Region**	**Left/Right**	**x**	**y**	**z**	**BA**	**^{k}E**	**t**
Two sample T-Test																
M1 C410Y > Controls									**Controls > M1 C410Y**							
Associative learning									***Associative learning***							
no significant differences									Hippocampus	R	26	-10	-22		17	4.56
										R	26	-30	-6		5	3.98[a]
									Amygdala/Hippocampus	L	-32	-8	-26		11	4.74[a]
									Middle frontal gyrus	L	-24	60	-2	10	72	7.45
										R	24	60	8	10	31	4.92
									Superior temporal gyrus	L	-56	-2	-4	22	101	6.16
										L	-50	-20	4	22	92	6.1
										R	52	-8	-4	22	38	4.29
									Cingulate gyrus	R	2	38	6	24	35	3.58
									Fusiform gyrus	L	-32	-36	-30	36	26	4.65
Single face learning									***Single face learning***							
Middle frontal gyrus	R	26	52	-10	11	24	5.77		(Hippocampus)	L	-24	-34	2		56	3.95[a]
									Inferior frontal gyrus	R	44	38	-10	47	41	4.78
									Middle frontal gyrus	L	-20	60	-2	10	123	8.11
										R	18	62	2	10	127	5.04
									Medial frontal gyrus	L	-10	48	-6	10	29	5.17
									Orbital gyrus	L	-28	46	-14	11	190	9.29
									Superior temporal gyrus	L	-36	8	-28	22	257	7.74
										L	-56	0	-4	22	113	5.73
										L	-58	-10	4	22	196	5.57
Associative retrieval									***Associative retrieval***							
Orbital gyrus	R	18	54	-10	11	22	6.07		Hippocampus	L	-22	-30	-10		10	4.07[a]
										R	24	-10	-20		25	3.68[a]
									Parahippocampal gyrus	R	30	-32	-14		39	3.81[a]
									Entorhinal cortex	L	-16	-4	-32		19	5.43
									Superior temporal gyrus	L	-52	-2	-6	22	51	5.38
										R	62	-8	4	22	18	4.36
Face recognition									***Face recognition***							
no significant differences									Hippocampus	R	20	-30	-12		28	4.86
									Parahippocampal gyrus	L	-12	-36	-6		81	4.61
									Middle frontal gyrus	L	-22	60	-2	10	16	5.62
New face detection									***New face detection***							
no significant differences									Hippocampus	R	20	-30	-12		26	3.59[a]
									Superior temporal gyrus	L	-54	0	-6	22	197	6.08
										L	-58	-10	8	22	43	4.82
									Medial frontal gyrus	R	8	-14	64	6	48	5.13
									Cerebellum	L	-32	-46	-32		46	4.28
Working memory									***Working memory***							
Inferior parietal lobule	R	54	-44	36	40	83	5.82		no significant differences							
Superior parietal lobule	R	32	-78	42	7	69	6.03									
Orbital gyrus	R	24	48	-12	11	65	6.43									
Precentral gyrus	R	54	2	6	6	44	5.04									

t: values of peaks within significantly activated clusters of voxels, kE: cluster size (in voxels), BA: Brodman area, a: $p < 0.005$

Table S6 Brain activation differences between subject M2 and the control group during the memory tasks

		MNI Coordinates (mm)								MNI Coordinates (mm)						
Brain Region	**Left/Right**	**x**	**y**	**z**	**BA**	**^{k}E**	**t**	**Brain Region**	**Left/Right**	**x**	**y**	**z**	**BA**	**^{k}E**	**t**
Two sample T-Test															
M2 > Controls								**Controls > M2**							
Associative learning								***Associative learning***							
Superior frontal gyrus	L	-18	-14	60	6	33	4.69	Hippocampus	R	36	-28	-14		48	5.8
Superior temporal gyrus	R	46	-28	4	22	44	5.36		R	22	-28	-12		5	4.33[a]
Fusiform gyrus	R	-20	-74	-8	19	32	5.43	Parahippocampal gyrus	L	-14	-44	-4	30	624	7.77
								Superior temporal gyrus	R	44	2	-24	22	23	5.2
Single face learning								***Single face learning***							
no significant differences								no significant differences							
Associative retrieval								***Associative retrieval***							
no significant differences								Hippocampus	R	34	-30	-12		95	5.17
									L	-26	-34	-8		14	4.24[a]
								Middle frontal gyrus	L	-40	10	54	6	71	4.93
								Middle temporal gyrus	R	48	10	-26	21	74	5.27
								Cingulate gyrus	L	-6	-46	14	30	17	5.03
Face recognition								***Face recognition***							
no significant differences								Middle frontal gyrus	R	34	28	26	46	16	4.59
								Inferior parietal lobule	R	48	-54	44	40	16	4.21
New face detection								***New face detection***							
no significant differences								Hippocampus	R	34	-30	-12		14	3.64[a]
									R	24	-32	-8		8	3.15[a]
								Inferior frontal gyrus	L	-56	12	30	9	52	5.79
Working memory								***Working memory***							
Middle frontal gyrus	R	34	54	-14	11	50	5.55	no significant differences							
Supramarginal gyrus	R	52	-58	40	40	144	5.89								
Middle temporal gyrus	R	56	-64	2	37	19	4.4								
Insula	R	40	12	-2		18	4.07								

t: values of peaks within significantly activated clusters of voxels, kE: cluster size (in voxels), BA: Brodman area, a: $p < 0.005$,

Table S7 Brain volumes

	Y1 C410Y	Controls (n = 9 males)	Y2	Y3	M1 C410Y	M2	Controls (n = 13 females)
Grey matter	653.76	781.03 ± 79.09	636.42	539.45	624.69	588.17	638.45 ± 50.98
White matter	457.52	501.28 ±36.03	385.23	341.18	423.56	426.62	386.95 ± 32.65
L. hippocampus	2.86	2.73 ± 0.33	2.51	2.55	2.54	2.63	2.52 ± 0.23
R. hippocampus	2.85	2.77 ± 0.30	2.56	2.47	2.70	2.58	2.50 ± 0.23
L. parahipp. gyr.	5.73	5.57 ± 0.61	4.17	4.83	5.16	6.90	5.02 ± 0.62
R. parahipp. gyr.	6.43	5.61 ± 0.51	4.91	4.41	6.13	6.36	4.87 ± 0.63

Values: means ± SD (ccm^3), L: left, R: right, parahipp. gyr.: parahippocampal gyrus

4. General Discussion

4.1. Aims of the present studies and some methodological remarks

The two studies presented here sought to detect genetic influences on memory capacity, memory-related brain activity, and neuroanatomy. We choose to investigate two AD-related genes, because they have already been implicated in memory functions in studies with MCI, prodromal AD, and AD patients. Moreover, the genetic variation in these genes has been associated with specific physiological effects at the cellular level and their impact has been described in distinct brain regions. First, we examined the apolipoprotein E gene, of which the ε4 allele is a known genetic riskfactor for AD. It exerts detrimental effects on memory performance, brain metabolism, and memory-related brain activity in middle-aged and elderly subjects, probably through its relation to the neuropathological hallmarks of the disease. Second, we examined the presenilin 1 gene, which is directly linked to the disease because of its causative nature. It is, however, conceivable that both genes might also play a role in normal interindividual memory variability, independently of their strong coupling to the Alzheimer's Disease causing neuropathological events.

To address our hypotheses (please see chapter 2) we combined genetic analyses, neuroimaging, quantitative neuropsychological assessments, and methods from experimental psychology. At the core of both studies was a memory-paradigm that included the learning and retrieval of face-profession associations and single faces. This paradigm had been chosen primarily to activate medial temporal lobe structures, including the hippocampus. These structures have been implicated in relational memory (Cohen and Eichenbaum, 1993; Gabrieli, 1998; Henke et al., 1997; Henke et al., 1999b). They are the first to be affected by neurofibrillary tangle formation, one of the hallmarks of AD (Braak and Braak, 1996; Delacourte et al., 1999). Neurofibrillary tangle formation and amyloid plaque deposition (the other neuropathologic hallmark of AD) lead to neural dysfunction and neural death, hence evoking memory decline at the beginning of the disease and a subsequent total memory loss in later stages of the disease. Our group showed in preceding studies that the tasks adopted here reliably activated the hippocampal formation (Degonda et al., 2005; Henke et al., 2003). For the present studies we adapted and extended past memory paradigms. Because we expected to detect only small genotype-induced changes in memory activity, the learning procedure was repeated three times. The repeated learning procedure allowed us to measure subtle repetition effects and turned out to be a great gain in the APOE study, where the most

significant genotype-induced brain activity changes could be tackled during repeated learning. Moreover, a working memory task and an attentional task were added, allowing us to investigate whether genotype induced effects on memory function were specific to episodic and semantic memory, or whether they also encompassed additional cognitive functions, like working memory or attentional abilities.

The investigation of genetic traits on memory functions imposes a quasi-experimental design. This means, that the genetic variation between subjects defines the independent variable, while the various measured memory functions that were modulated by the genotype define the dependent variable. Quasi-experimental designs imply the disadvantage of confounding variables. These can bias the measured effect on the independent variable. The contribution of single genes to the response characteristics of brain structures is presumably small. Typically large effects of age, gender and IQ as well as environmental factors such as illness, injury or substance abuse on phenotypic variance obscure the small potential gene effects. We therefore had to carefully match our samples in both studies for confounding variables known to have a modulatory effect on memory capacity, memory-related brain activity and neuroanatomy. Because our imaging protocol involved performance of a task, groups were also matched for level of performance. This is a crucial point because task performance and neural responses are tightly linked, and systematic differences in performance between genotype groups could have occluded a true gene effect. The careful matching of our subjects ensured that resulting differences between genotype groups (APOE study) or between mutation carriers and control subjects (PSEN1 study) could directly be related to the APOE genotype or the PSEN1 C410Y mutation, respectively.

4.2. Beneficial effects of the APOE ε4 allele on memory

In the APOE study we first examined associations between allelic variations in the APOE gene and memory performance in a sample of 340 healthy young adults. The ε4 allele, a genetic risk factor for the development of late-onset Alzheimer's Disease (Saunders et al., 1993), was associated with a significantly better performance in recalling words after a delay of 5 minutes, as compared to the ε2 and ε3 alleles. So far, most studies investigating effects of APOE on biological processes were conducted either in middle-aged to elderly nondemented healthy subjects or in prodromal AD patients. These studies suggested a detrimental role of APOE4 on memory performance (Baxter et al., 2003; Bondi et al., 1995; Hyman et al., 1996),

executive functions (Rosen et al., 2005), attention (Greenwood et al., 2000), memory-related brain activity (Bondi et al., 2005; Bookheimer et al., 2000), and brain glucose metabolism (Reiman et al., 2002b). Our finding of better memory in APOE4 carriers was, therefore, somewhat intriguing. However, it is conceivable, that APOE-related alterations in cerebral physiology may exist even from young age. Studies with very young subjects or even children documented effects of the APOE genotype on cognitive performance in type I diabetes (Ferguson et al., 2003), lipid metabolism (Srinivasan et al., 2001), blood pressure (Katsuya et al., 2002), atherosclerosis (Hixson, 1991), ischaemic heart disease (van Bockxmeer and Mamotte, 1992), and myocardial infarction (Brscic et al., 2000). Moreover, evidence from studies with young mice indicates, that the APOE genotype affects stress response and spatial memory (Zhou et al., 1998), and regulates synaptic plasticity and long term potentiation in the hippocampus (Valastro et al., 2001).

In a second step, we examined 34 subjects of the large sample group with fMRI to investigate, on a neurophysiologic level, the advantageous APOE4-effects on memory that we had found in the large sample. The APOE4 allele was associated with less learning- and retrieval-related brain activity compared to APOE2 and APOE3. The smaller hippocampal and neocortical activity was found at comparable performance levels in the memory tasks between the three genotype groups (i.e. APOE ε2/ε3, ε3/ε3, and ε3/ε4). Also, neuropsychological test performance and brain volumes did not significantly differ between groups. Therefore, the measured signal differences between the three APOE groups were neither related to differences in cognition nor to brain morphology alterations, but reflected an unbiased APOE genotype effect on memory-related neurophysiologic functions. The most intriguing finding, however, was revealed during repeated learning. While the APOE4 carriers decreased learning-related hippocampal and neocortical activity, the APOE4 noncarriers increased their learning-related activity. Moreover, decreasing learning-related activity denoted the good performers in the APOE4 group, while increasing learning-related activity denoted the good performers in the APOE2 group. We did not find a significant correlation of medial temporal activity slopes with the shortening of reaction latencies during repeated learning in any APOE group. Therefore the genotype-dependent brain activity differences likely reflected a differential neurophysiology underlying episodic memory and not another form of memory (i.e. priming) which was simultaneously active during the learning runs.

To our knowledge this is the first study that investigated APOE genotype effects on normal human memory variability using functional magnetic resonance imaging. The results suggest that the APOE4 allele is associated with a better memory and a more economic use of

memory-related neural resources in young and healthy subjects. Our findings of an advantageous effect of APOE4 early in life add to a variety of studies showing beneficial effects of APOE4 in young humans (Hubacek et al., 2001; Ravaja et al., 1997; Yu et al., 2000; Zetterberg et al., 2002) and young animals (Kitamura et al., 2004; Ohkubo et al., 2001). Nevertheless, the beneficial effect of the APOE4 allele stands in contrast to most studies reporting an association of APOE4 with detrimental effects on cognition and brain functions in middle-aged and elderly human subjects. Although our findings are, with high probability, not related to AD pathology, it is still unclear whether the brains of young adults are completely devoid of such depositions 30-50 years prior to the statistical onset of the disease. A neuropathological study examined 105 necropsy cases which showed no signs of dementia. Abnormally high brain ß-amyloid levels were reported for ε4 carriers as young as 40 years of age, suggesting that APOE4 carriers begin to accumulate ß-amyloid earlier in life than non-carriers. However, neurofibrillary tangle formation is rarely occurring in the brains of 20-30 years olds (Ghebremedhin et al., 1998), whereas brain amyloid deposition has been found irrespective of APOE genotype in young subjects (Morishima-Kawashima et al., 2000). Therefore, it is unlikely that early neuropathological changes underlie our fMRI results.

Our study was not able to answer the question, whether the beneficial effect of APOE4 on memory in young adulthood and the detrimental effects in later stages of life are two distinct processes running parallely along each other, or alternatively, whether there might be a transition point between 20 and 40 years of age, where initial beneficial effects start to turn into lethality. Possibly, this question can not be answered by means of neuroimaging alone, but must be investigated at the cellular level. However, Reiman et al. (2004) used FDG PET in cognitively healthy young (20-39 years) APOE4 carriers to detect earliest AD-pathology induced changes in glucose metabolism. They found abnormally low rates of glucose metabolism bilaterally in the posterior cingulated, parietal, temporal, and prefrontal cortex, brain regions known to be affected by neuropathological changes in AD. These results suggest that glucose metabolism could be the consequence of a very limited histopathology that may or may not be present in cognitively normal young adults. However, the authors argue that, alternatively, this hypometabolism could also reflect abnormalities in prenatal or early postnatal neurological development.

A longitudinal follow-up of our subjects in the next ten years would render valuable information about how memory-related brain activity and memory performance is modulated by APOE4 during a period in witch presumably AD-related neuropathologic deposits start to

accumulate. Hopefully one could detect time windows in which changes from smaller (more economic) brain activity to brain activity with greater magnitude and extent (compensatory activity) are observable, hence determining earliest preclinical signs of AD.

Although our study delivers first insights into the involvement of the APOE gene in normal human memory variability on the behavioural and neurophysiological level, future studies need to investigate what the underlying cellular mechanisms are. Profound knowledge of the effects of APOE isoforms on memory mechanisms will not only broaden basic knowledge about memory function in general, but could also open the way for a detection of AD 30-50 years prior to its clinical onset.

4.3. Influence of the PSEN1 C410Y mutation on memory – earliest preclinical signs of AD

In this study we investigated five members of a family with FAD. Two of the family members, a young man (20 years old) and a middle-aged woman (45 years old) carried the C410Y mutation on the PSEN1 gene. This mutation is transmitted in an autosomal dominant manner and leads to familial Alzheimer's Disease with a reported age of onset of 48 years (Alzheimer's Disease Collaborative Group, 1995).

As yet the earliest neuropsychological features of FAD have been found in individuals at risk of autosomal dominant FAD within one decade prior to the clinical onset of the disease (Fox et al., 1998; Ringman et al., 2005). Attempts to detect early signs of the disease have also been made by the use of neuroimaging techniques (Bookheimer et al., 2000; Gregory et al., 2005; Reiman et al., 2004; Reiman et al., 1996; Rossor et al., 1996; Small et al., 1995). AD-related neuropathologic depositions start 15-30 years prior to the onset of the disease (Davies et al., 1988; Rumble et al., 1989) and subsequently lead to neuronal loss. It is therefore conceivable that neural dysfunction might be reflected in the measured brain signals decades prior to the clinical manifestation.

Here we were able to show that memory deficits can be detected as long as 30 years prior to the onset of FAD. The two PSEN1 C410Y mutation carriers examined in this study showed selective episodic memory impairments in neuropsychological memory tests. The young mutation carrier presented with visual episodic memory impairments that exceeded two standard deviations as compared to an age- and education-matched control population. In all other tests the young mutation carrier performed well and showed scores of high intelligence.

The memory impairments in the middle-aged mutation carrier were further progressed, encompassing both verbal and visual memory. These data suggest that the middle-aged mutation carrier was suffering from mnestic mild cognitive impairment (Petersen et al., 2001) at the time of examination.

We also compared both mutation carriers to family members without the mutation. A young mutation non-carrier showed low neuropsychological scores on a wide range of cognitive functions and a low intelligence. Most likely the low scores in this subject can be attributed to her low inherent cognitive capacity. The middle-aged mutation non-carrier only showed a low performance in tests of intelligence. These results emphasize the selectivity of the episodic memory impairments in the mutation carriers and relate them to the PSEN1 C410Y mutation. However, the selective episodic memory impairments might not reflect an influence of the mutation on normal neurophysiologic function, but rather reflect a detrimental effect of PSEN1 through AD-related neuropathology. The selective memory impairment in the young mutation carrier is most likely attributable to neural dysfunction in the MTL, evoked by early neurofibrillary tangle formation (Braak and Braak, 1996) and amyloid plaque deposition. Neurofibrillary tangle formation starts in the entorhinal cortex (Braak and Braak, 1996), thereby making the MTL the most vulnerable brain area for early preclinical memory impairments.

MTL dysfunction in our young mutation carrier is most likely explainable through the direct effects of the presenilin 1 protein on tau phosphorylation or through an indirect effect on tau-pathology (Boutajangout et al., 2002; Takashima et al., 1998). PSEN1 mutations have been associated with significant increases of plasma Aß42 levels together with a massive deposition of Aß42 in the brain (Iwatsubo, 1998; Lemere et al., 1996). The accumulation of Abeta appears to be an early and initiating event that triggers a series of downstream processes including misprocessing of the tau protein (St George-Hyslop and Petit, 2005). It is conceivable that our young mutation carrier suffers from early neuropathologic deposits, because the genetic defect is present since birth. This notion is supported by studies of patients with Down's syndrome[10] where deposition of Aß 1-42 has been observed as early as in the third decade (Stoltzner et al., 2000; Teller et al., 1996) of life.

[10] Down's syndrom (trisomy 21) is a genetic disorder which is characterized by the presence of a third chromosome 21 (including the Aß-amyloid precursor protein gene). Subjects with Down's syndrome over the age of 40 years show AD-like neuropathological and neurochemical abnormalities post-mortem. In these patients, cognitive dysfunctions and dementia increase with aging, and more than 75% over 60 are demented (Lai and Williams, 1989). Therefore, Down's Syndrome is a unique human model with which preclinical stages of AD and the transition to dementia can be studied (Holland and Oliver, 1995).

Detrimental effects of the PSEN1 C410Y mutation were also reflected in brain signal measures with fMRI. Here both mutation carriers exhibited significantly altered patterns of memory-related brain activity as compared to the control group and the family members without the mutation. The young mutation carrier showed enhanced brain activity in the hippocampus and a memory-related network during learning and retrieval of face-profession associations and single faces. This amplified activity was almost only attributable to structures of the left hemisphere, and appeared at a comparable performance level between the young mutation carrier and the control group in the fMRI memory tasks. The most likely explanation for the task-related activity enhancements in the MTL and the neocortical regions is a compensatory reponse to accumulating AD pathology. The neuropsychology data suggest that the young mutation carrier is suffering from right-lateralized neural dysfunction in the MTL and neocortical areas because he showed selective visual memory impairments in nonverbal tasks. It is, therefore, likely that the left hemisphere has to 'work harder' to overcome memory deficits which are the consequence of the right-lateralized AD-related neuropathology. These results are in line with studies that showed task-related brain signal amplifications in the MTL and neocortical structures in elderly non-demented AD risk allele (APOE4) carriers (Bondi et al., 2005; Bookheimer et al., 2000). The 'compensatory hypothesis' proposed in these studies suggests that after an initial decline in memory proficiency following damage to MTL structures, patients in the preclinical stage of AD are able to effectively recruit compensatory brain resources to halt or slow further memory decline for a period of time.

The middle-aged mutation carrier, on the other hand, showed a low performance in the face recognition task and exhibited almost only reduced activity in the MTL and in memory-related structures while performing the memory-tasks. Decreased task-induced brain activity in early stage AD patients has been found as well as compensatory amplification of task-induced brain activity in MCI patients (Buckner et al., 2000; Dickerson et al., 2004; Kato et al., 2001; Rosano et al., 2005; Small et al., 1999). In our subject, the decreased activity most likely reflects gross neural dysfunction that is a consequence of the further progressed accumulation of amyloid deposits and neurofibrillary tangle formation. The degree of neural dysfunction in FAD is much more extensive than in the sporadic AD cases, and might therefore have extensively diminished still functioning neural ensembles that would have allowed a compensatory response in the middle-aged mutation carrier.

Both mutation carriers could be clearly differentiated from the control groups and the family members without the mutation in terms of the observed memory-related brain activity

patterns. While the family members without the mutation showed a balanced pattern of memory-related activity (i.e. they showed areas of increased as well as areas of reduced activity in comparison to the control group) this was not the case for the young and the middle-aged mutation carriers. They either showed enhanced or reduced memory-related brain activity. This suggests that the brain activity alterations in the mutation carriers were selectively modulated by the PSEN1 mutation. Moreover, the effect of the mutation was only evident in tasks of episodic memory. In the working memory experiment, each and every family member showed enhanced brain activity as compared to the control group. This 'family pattern' of enhanced activity occurred independently of genotype, hence confirming the role of PSEN1 in episodic memory function.

Interestingly, we did not find alterations in brain morphology induced by the PSEN1 mutation. It has been shown, that FAD-causing mutations on the PSEN1 gene and the APP gene can influence the degree and the regional pattern of atrophy (Gregory et al., 2005). Moreover these mutations were associated with greater medial temporal lobe atrophy in cases with FAD than in sporadic AD cases, suggesting that mutations affecting Aß metabolism selectively increase hippocampal degeneration. Also, cases with mutations in the PSEN1 gene demonstrated additional increased frontotemporal atrophy. In our young mutation carrier early neuropathological events might have led to neural dysfunction (as reflected in memory performance and memory-related brain activity), but not yet to neural death. Therefore, morphological changes in the young mutation carrier would have been unlikely. In the middle-aged mutation carrier, however, progressed accumulation of amyloid plaques and neurofibrillary tangle formation might have led to neuronal loss. Possibly, neuronal death in that subject was only of a small extent and therefore did not lead to detectable gross morphological changes before the clinical onset of FAD.

Taken together, these results suggest that early preclinical signs of AD are detectable three decades before the onset of the disease on the behavioral and the neural level. However, our results must remain tentative because they are based on a single case. Therefore we cannot generalize findings to other PSEN1 mutations or to sporadic cases with probable AD. Still, one can be confident that with a future improvement of fMRI image quality through higher magnetic fields and with the application of sensitive episodic memory tasks tailored to hippocampal function subtle changes in MTL-function might be detectable decades before the onset of AD, possibly even in sporadic cases. The preclinical detection of earliest markers for AD is the prerequisite for a hopefully effective preventive treatment of the disease, and therefore remains a core issue in future AD research.

5. Conclusions

The recent completion of the human genome sequence provides unprecedented opportunities to explore the genetic basis of individual differences in complex behaviours and the vulnerability to neuropsychiatric disorders. Linkage and association analyses were the traditional methods of choice for finding susceptibility genes for complex disorders. However, they did not provide information about the mechanisms by which such genes modulate normal physiologic functions or increase biological risks for neuropsychiatric diseases. Traditionally, the impact of genetic polymorphisms has been examined on the behavioural level using personality questionnaires and neuropsychological batteries. Because of the individual variability and subjectivity of such behavioural measures it was necessary to use very large samples (i.e. several hundred subjects) to identify even small gene effects (Glatt and Freimer, 2002). In such studies potential gene effects on the task-related neural substrates might have been obscured, for example, because of the interindividual applied alternative task strategies in the behavioral tests. Functional genetic polymorphism may have a more robust impact on the neural level than on the behavioural level and therefore neural responses related to specific cognitive and emotional processes may be more objectively measurable by means of non-invasive neuroimaging techniques like fMRI. In comparison to pure behavioural assessments, neuroimaging techniques require considerably fewer subjects to identify significant gene effects on the response characteristics of the brain and allow the investigation of the specificity of gene effects by examining their influence on multiple functional neural systems in a single subject. Due to these advantages neuroimaging emerged as the method of choice for the *in vivo* study of functional genetic variations.

The results of the two studies presented here underscore the power of functional magnetic resonance imaging to identify functional polymorphisms in genes that are likely important either for normal human memory or for pathological memory alterations in the context of a neuropsychiatric disease (i.e. Alzheimer's Disease). They also show the advantage of a systems level approach by integrating genetic information with a phenotypic trait (i.e. cognitive, neurophysiological, and neuroanatomical measures) to successfully determine the influence of genes on brain function.

We used functional and structural MRI to determine the impact of the APOE gene and the PSEN1 gene on memory performance, memory-related brain activity and neuroanatomy. We were able to show, that APOE4, an allele associated with detrimental effects on several biological processes and with an increased risk for the development of AD, exerted beneficial

effects on memory function in young and healthy adults. These effects were likely not related to AD-neuropathology, and therefore suggest that the APOE gene plays an important role in normal memory function, independent of its relation to pathological mechanisms of AD.

The mutation on the PSEN1 gene, on the other hand, was associated with detrimental effects on memory performance and memory-related brain activity in a young mutation carrier, most likely suggesting that PSEN1 mutation effects on memory were mediated through the neuropathology of AD. Because PSEN1 mutation carriers bear the mutation since birth, it is likely that AD-related neuropathological events take place already in the early years of life. It was therefore almost impossible to measure an AD pathology independent effect of the PSEN1 mutation on normal memory function in this young subject.

Nevertheless, both studies provide useful insights into how genetic variants modulate memory functions on the neural and the behavioural level. Our results complete findings from previous studies that reported an influence of polymorphisms of the 5HT-2a receptor encoding gene, the PRNP gene, and the BDNF gene on normal human memory function (deQuervain et al., 2003; Papassotiropoulos et al., 2005; Egan et al., 2003; Hariri et al., 2003) Moreover, our findings might be implicated in future AD research aiming at the preclinical detection of earliest markers of the disease.

The genetic basis of human cognition, behaviour, and psychiatric illnesses is of a high complexity with each gene exerting only a small effect. A single gene can affect multiple processes, multiple genes can impact on a single process, and multiple cognitive processes are intercorrelated. The association between a genotype and a variation in a cognitive function can not be taken as a proof for the impact of the genetic variant on brain function, because many confounding factors could account for the association. To overcome this problem, we thoroughly minimized potentially confounding factors in our studies and accounted for possible genetic heterogeneity between subjects. Therefore, one can be confident that the results in our studies reflect true genetic effects on cognition and brain function.

So far, the majority of studies that used neuroimaging to investigate the genetic basis of memory reported only effects of single genetic polymorphisms. Future studies need to investigate the functional interactions between multiple gene variants (gene clusters), the environment, and their collective impact on brain function. Also, it is conceivable that molecular mechanisms underlying genetic influences on neural information processing might be detected.

Finally, to determine a comprehensive genetic basis of human cognition the quest for candidate genes must be extended to other important human cognitive domains, including basic aspects of language, attention or visual processing.

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Curriculum vitae

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Born:	May 20th, 1974, Schlieren, Switzerland	
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Spoken languages :	German, Italian, English, (French)	

Education

1981-1987	Primary school
1987-1990	Secondary school
1990-1995	High school (Wirtschaftsmatur Typus E, Kantonsschule Enge, Zürich)
1995-1996	Study of materials engineering at the Federal Institute of Technology (ETH) Zurich (6 months)
1996-2002	Study of psychology at the University of Zurich

Main subject:	Experimental psychology
1. subsidiary subject:	Neurophysiology
2. subsidiary subject:	Informatics

July-Sept. 2000	Internship at the Division of Psychiatry Research, University of Zurich. The internship consisted of a clinical part, namely the examination of patients with dementia and the differential diagnosis of dementia. The other part of the internship consisted of research on pilot studies of Dr. Katharina Henke's fMRI study *„Medial temporal activation during nonconscious retrieval of new semantic associations"*.
November 2002	Masters degree in psychology (Licentiatus philosophiae). Title of Master Thesis: 'Spielt das hippokampale Areal für das nicht-bewusste Erinnern von neuen semantischen Assoziationen eine Rolle? Ein Studie mit funktioneller Magnetresonanzbildgebung (fMRI).

December 2002	Beginning of PhD Studentship/Program in Neuroscience at the Division of Psychiatry Research, University of Zurich & at the Center of Neuroscience, University of Zurich
December 2005	Philosophical Doctor degree (PhD). Title of the doctoral thesis: 'Genetic Influences on Memory Performance, Memory-Related Brain Activity, and Neuroanatomy Investigated with Functional and Structural Magnetic Resonance Imaging'.

Continuing Education

1996	English language course in London (4 weeks).
January 2003	'Functional Brain Imagery Of Cognitive Processes: Methods and Statistical Analyses'. Course at the University of Geneva.
Februray 2003	'Statistical Parametric Mapping (SPM) in Zurich'. Course at the University of Zurich.

Teaching experience

2000	Tutor for the lecture Neurophysiology II, held by Prof. Dr. Marie-Claude Hepp-Reymond, at the University of Zurich (one semester).

Publications

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